

## PATENT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner  
 US Department of Commerce  
 United States Patent and Trademark  
 Office, PCT  
 2011 South Clark Place Room  
 CP2/5C24  
 Arlington, VA 22202  
 ETATS-UNIS D'AMERIQUE  
 in its capacity as elected Office

|   |   |
|---|---|
| <b>Date of mailing (day/month/year)</b><br>06 March 2001 (06.03.01)         |   |
| <b>International application No.</b><br>PCT/JP00/03334                      | <b>Applicant's or agent's file reference</b><br>09364           |
| <b>International filing date (day/month/year)</b><br>24 May 2000 (24.05.00) | <b>Priority date (day/month/year)</b><br>28 May 1999 (28.05.99) |
| <b>Applicant</b><br>MATSUOKA, Nobuya et al                                  |   |

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:  
20 December 2000 (20.12.00)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was  
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

|   |  |
|---|--|
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland<br>Facsimile No.: (41-22) 740.14.35 | Authorized officer<br>R. Forax<br>Telephone No.: (41-22) 338.83.38 |
|---|--|

## PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING  
OF A CHANGE(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

To:

TAKASHIMA, Hajime  
Fujimura Yamato Seimei Bldg.  
2-14, Fushimimachi 4-chome, Chuo-ku  
Osaka-shi, Osaka 541-0044  
JAPON

|   |  |
|---|--|
| Date of mailing (day/month/year)<br>16 August 2001 (16.08.01) | <b>IMPORTANT NOTIFICATION</b>  |
| Applicant's or agent's file reference<br>09364                |  |
| International application No.<br>PCT/JP00/03334               | International filing date (day/month/year)<br>24 May 2000 (24.05.00) |

## 1. The following indications appeared on record concerning:

☐ the applicant      ☐ the inventor      ☒ the agent      ☐ the common representative

|  |                      |                    |
|--|----------------------|--------------------|
| Name and Address<br>TAKASHIMA, Hajime<br>Yuki Bldg.<br>3-9, Hiranomachi 3-chome, Chuo-ku<br>Osaka-shi, Osaka 541-0046<br>Japan | State of Nationality | State of Residence |
|  | Telephone No.        |                    |
|  | Facsimile No.        |                    |
|  | Teleprinter No.      |                    |

## 2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person      ☐ the name      ☒ the address      ☐ the nationality      ☐ the residence

|  |                      |                    |
|--|----------------------|--------------------|
| Name and Address<br>TAKASHIMA, Hajime<br>Fujimura Yamato Seimei Bldg.<br>2-14, Fushimimachi 4-chome, Chuo-ku<br>Osaka-shi, Osaka 541-0044<br>Japan | State of Nationality | State of Residence |
|  | Telephone No.        |                    |
|  | Facsimile No.        |                    |
|  | Teleprinter No.      |                    |

## 3. Further observations, if necessary:

## 4. A copy of this notification has been sent to:

|   |   |
|---|---|
| <input checked="" type="checkbox"/> the receiving Office                              | <input type="checkbox"/> the designated Offices concerned         |
| <input checked="" type="checkbox"/> the International Searching Authority             | <input checked="" type="checkbox"/> the elected Offices concerned |
| <input checked="" type="checkbox"/> the International Preliminary Examining Authority | <input type="checkbox"/> other:                                   |

|   |   |
|---|---|
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland | Authorized officer<br><br>Masashi HONDA |
| Facsimile No.: (41-22) 740.14.35  | Telephone No.: (41-22) 338.83.38        |

## PATENT COOPERATION TREATY

PCT

NOTIFICATION CONCERNING  
SUBMISSION OR TRANSMITTAL  
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

From the INTERNATIONAL BUREAU

To:

TAKASHIMA, Hajime  
Yuki Bldg.  
3-9, Hiranomachi 3-chome, Chuo-ku  
Osaka-shi, Osaka 541-0046  
JAPON

|  |  |
|--|--|
| Date of mailing (day/month/year)<br>19 September 2000 (19.09.00)     | <b>IMPORTANT NOTIFICATION</b>  |
| Applicant's or agent's file reference<br>09364                       |  |
| International application No.<br>PCT/JP00/03334                      | International filing date (day/month/year)<br>24 May 2000 (24.05.00) |
| International publication date (day/month/year)<br>Not yet published | Priority date (day/month/year)<br>28 May 1999 (28.05.99)             |
| Applicant<br>FUJISAWA PHARMACEUTICAL CO., LTD. et al                 |  |

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.
3. An asterisk(\*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
4. The letters "NR" appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

| <u>Priority date</u>   | <u>Priority application No.</u> | <u>Country or regional Office<br/>or PCT receiving Office</u> | <u>Date of receipt<br/>of priority document</u> |
|------------------------|---------------------------------|---|---|
| 28 May 1999 (28.05.99) | 09/321,745                      | US  | 11 Augu 2000 (11.08.00)                         |

|  |   |
|--|---|
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland<br><br>Facsimile No. (41-22) 740.14.35 | Authorized officer<br><br>Carlos Naranjo<br><br>Telephone No. (41-22) 338.83.38 |
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# PATENT COOPERATION TREATY

WO 00/72834  
PCT/JP00/03334

**PCT**

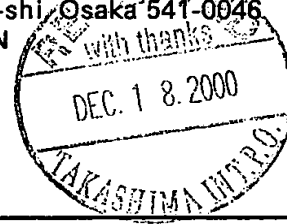
## NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

From the INTERNATIONAL BUREAU

To:

TAKASHIMA, Hajime  
Yuki Bldg.  
3-9, Hiranomachi 3-chome, Chuo-ku  
Osaka-shi, Osaka 541-0046  
JAPON



|   |  |  |
|---|--|--|
| Date of mailing (day/month/year)<br>07 December 2000 (07.12.00) |  |  |
| Applicant's or agent's file reference<br>09364                  |  | <b>IMPORTANT NOTICE</b>                                  |
| International application No.<br>PCT/JP00/03334                 | International filing date (day/month/year)<br>24 May 2000 (24.05.00) | Priority date (day/month/year)<br>28 May 1999 (28.05.99) |
| Applicant<br>FUJISAWA PHARMACEUTICAL CO., LTD. et al            |  |  |

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:  
**KR,US**

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:  
**BR,CA,CN,EP,JP**

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on 07 December 2000 (07.12.00) under No. WO 00/72834

### REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

### REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

|   |  |
|---|--|
| <p>The International Bureau of WIPO<br/>34, chemin des Colombettes<br/>1211 Geneva 20, Switzerland</p> <p>Facsimile No. (41-22) 740.14.35</p> | <p>Authorized officer<br/><br/>J. Zahra</p> <p>Telephone No. (41-22) 338.83.38</p> |
|---|--|

**Annex to Form PCT/ISA/206  
COMMUNICATION RELATING TO THE RESULTS  
OF THE PARTIAL INTERNATIONAL SEARCH**

International Application No  
**PCT/JP 00/03334**

1. The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the results of the international search established on the parts of the international application which relate to the invention first mentioned in claims Nos.:  
**1-28, 37**
2. This communication is not the international search report which will be established according to Article 18 and Rule 43.
3. If the applicant does not pay any additional search fees, the information appearing in this communication will be considered as the result of the international search and will be included as such in the international search report.
4. If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on other parts of the international application for which such fees will have been paid.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.                                |
|------------|--|--|
| X          | MATSUOKA, NOBUYA ET AL: "FK960<br>'N-(4-acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate!, a novel potential<br>antidementia drug, improves visual<br>recognition memory in rhesus monkeys:<br>comparison with physostigmine"<br>J. PHARMACOL. EXP. THER. (1997), 280(3),<br>1201-1209 ,<br>1997, XP001015389<br>abstract | 1-3,<br>6-10,<br>13-17,<br>20-24,<br>27,28,<br>33-38 |
| X          | GRAUL, A. ET AL: "FK-960. Cognition<br>enhancer"<br>DRUGS FUTURE (1997), 22(8), 830-832 ,<br>1997, XP001015394<br><br>page 830, column 2, paragraph 3 -page 832,<br>column 1, paragraph 2  | 1-3,<br>6-10,<br>13-17,<br>20-24,<br>27,28,<br>33-38 |
| X          | WO 98 25914 A (BABA YUKIHISA ;MIMURA<br>HISASHI (JP); FUJISAWA PHARMACEUTICAL CO<br>(JP) 18 June 1998 (1998-06-18)<br><br>page 1, line 1-22; claims 1,6,7<br><br>-/-   | 1-3,<br>6-10,<br>13-17,<br>20-24,<br>27,28,<br>33-38 |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |   |  |
|--|---|--|
| Category *   | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.  |
| X  | <p>DATABASE BIOSIS 'Online!<br/>BIOSCIENCES INFORMATION SERVICE,<br/>PHILADELPHIA, PA, US;<br/>1 June 1998 (1998-06-01)<br/>MATSUOKA NOBUYA ET AL: "FK960, a novel<br/>potential anti-dementia drug, augments<br/>long-term potentiation in mossy fiber-CA3<br/>pathway of guinea-pig hippocampal slices."<br/>Database accession no. PREV199800350951<br/>XP002176821<br/>abstract<br/>&amp; BRAIN RESEARCH,<br/>vol. 794, no. 2, 1 June 1998 (1998-06-01),<br/>pages 248-254,<br/>ISSN: 0006-8993</p> | <p>1-3,<br/>6-10,<br/>13-17,<br/>20-24,<br/>27,28,<br/>33-38</p> |
| E  | <p>WO 00 42011 A (AOKI SATOSHI ;YAMADA AKIRA<br/>(JP); FUJISAWA PHARMACEUTICAL CO (JP))<br/>20 July 2000 (2000-07-20)<br/>the whole document</p>  | <p>1-28,<br/>33-38</p>   |
| X  | <p>WO 98 35951 A (OHNE KAZUHIKO ;SHIMA ICHIRO<br/>(JP); FUJISAWA PHARMACEUTICAL CO (JP))<br/>20 August 1998 (1998-08-20)</p> <p>page 1, line 16 -page 4, line 13<br/>page 24, line 2-13<br/>claims 1-6; examples 1-27</p>   | <p>1-3,<br/>6-10,<br/>13-17,<br/>20-24,<br/>27,28,<br/>33-38</p> |
| X  | <p>EP 0 436 734 A (FUJISAWA PHARMACEUTICAL<br/>CO) 17 July 1991 (1991-07-17)</p> <p>abstract<br/>page 2, line 14-27; claims 1,3-7; examples<br/>1-6</p>   | <p>1-3,<br/>6-10,<br/>13-17,<br/>20-24,<br/>27,28,<br/>33-38</p> |
| X  | <p>WO 97 36871 A (WHITTLE PETER JOHN ;LOWE<br/>JOHN ADAMS III (US); PFIZER (US))<br/>9 October 1997 (1997-10-09)</p> <p>abstract<br/>page 11, line 3 -page 13, line 2; claims<br/>7,8; examples 3,10-13,25,42,81</p> <p style="text-align: center;">-/-</p>   | <p>1-3,<br/>6-10,<br/>13-17,<br/>20-24,<br/>27,28,<br/>33-38</p> |

Annex to Form PCT/ISA/206  
COMMUNICATION RELATING TO THE RESULTS  
OF THE PARTIAL INTERNATIONAL SEARCH

International Application No  
PCT/JP 00/03334

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.                           |
|------------|--|---|
| X,P        | DATABASE WPI<br>Section Ch, Week 200004<br>Derwent Publications Ltd., London, GB;<br>Class B02, AN 2000-052797<br>XP002176822<br>& WO 99 55674 A (DAINIPPON PHARM CO LTD),<br>4 November 1999 (1999-11-04)<br>table 7<br>abstract<br><br>----- | 1,2,4-9,<br>11-16,<br>18-23,<br>25-28,<br>33-38 |

# Patent Family Annex

Information on patent family members

International Application No

PCT/JP 00/03334

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s)   | Publication<br>date  |
|---|---------------------|--|--|
| WO 9825914 A                              | 18-06-1998          | AU 722095 B<br>AU 5136898 A<br>CN 1245495 A<br>EP 0944612 A<br>JP 2000514460 T<br>TW 422844 B<br>US 6147079 A  | 20-07-2000<br>03-07-1998<br>23-02-2000<br>29-09-1999<br>31-10-2000<br>21-02-2001<br>14-11-2000   |
| WO 0042011 A                              | 20-07-2000          | AU 1890400 A   | 01-08-2000   |
| WO 9835951 A                              | 20-08-1998          | EP 0968201 A   | 05-01-2000   |
| EP 0436734 A                              | 17-07-1991          | DE 69022965 D<br>DE 69022965 T<br>DK 436734 T<br>HK 64196 A<br>WO 9101979 A<br>JP 2531304 B<br>US 5250528 A  | 16-11-1995<br>04-04-1996<br>20-11-1995<br>19-04-1996<br>21-02-1991<br>04-09-1996<br>05-10-1993   |
| WO 9736871 A                              | 09-10-1997          | AP 677 A<br>AU 729129 B<br>AU 1554897 A<br>BG 102872 A<br>BR 9708386 A<br>CA 2250372 A<br>CN 1215391 A<br>CZ 9802614 A<br>EP 0891332 A<br>HR 970174 A<br>HU 9902455 A<br>JP 11510513 T<br>NO 984516 A<br>PL 329195 A<br>TR 9801930 T<br>US 6235747 B<br>ZA 9702689 A | 25-09-1998<br>25-01-2001<br>22-10-1997<br>30-11-1999<br>03-08-1999<br>09-10-1997<br>28-04-1999<br>11-08-1999<br>20-01-1999<br>31-12-2000<br>29-11-1999<br>14-09-1999<br>28-09-1998<br>15-03-1999<br>21-12-1998<br>22-05-2001<br>28-09-1998 |
| WO 9955674 A                              | 04-11-1999          | AU 3171699 A<br>BR 9909991 A<br>CN 1298391 T<br>EP 1076055 A<br>NO 20005430 A<br>JP 2000080081 A   | 16-11-1999<br>26-12-2000<br>06-06-2001<br>14-02-2001<br>20-12-2000<br>21-03-2000   |



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PATENT COOPERATION TREATY

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## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

|   |   |  |
|---|---|--|
| Applicant's or agent's file reference<br>09364  | <b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |  |
| International application No.<br>PCT/JP00/03334   | International filing date (day/month/year)<br>24/05/2000  | Priority date (day/month/year)<br>28/05/1999 |
| International Patent Classification (IPC) or national classification and IPC<br>A61K31/00 |   |  |
| Applicant<br>FUJISAWA PHARMACEUTICAL CO., LTD. et al.                                     |   |  |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.


2. This REPORT consists of a total of 7 sheets, including this cover sheet.

- ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

|   |  |
|---|--|
| Date of submission of the demand<br><br>20/12/2000  | Date of completion of this report<br><br>15.02.2002                      |
| Name and mailing address of the international preliminary examining authority:<br><br> European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer<br><br>Beeck, M<br><br>Telephone No. +49 89 2399 8473 |



# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/JP00/03334

## I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

**Description, pages:**

1-57 as originally filed

**Claims, No.:**

1-38 as originally filed

**Drawings, sheets:**

1/4-4/4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/JP00/03334

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

## III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 8-14.

because:

☒ the said international application, or the said claims Nos. 8-14 relate to the following subject matter which does not require an international preliminary examination (*specify*):  
**see separate sheet**

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 29-32.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

## IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/JP00/03334

- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☐ neither restricted nor paid additional fees.
2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
- ☐ complied with.
- ☒ not complied with for the following reasons:  
**see separate sheet**
4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:
- ☐ all parts.
- ☒ the parts relating to claims Nos. 1-28,33-38.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

|                               |      |        |                                  |
|-------------------------------|------|--------|----------------------------------|
| Novelty (N)                   | Yes: | Claims | 4,5,11,12,18,19,25,26            |
|                               | No:  | Claims | 1-3,6-10,13-17,20-24,27,28,33-38 |
| Inventive step (IS)           | Yes: | Claims |                                  |
|                               | No:  | Claims | 1-28,33-38                       |
| Industrial applicability (IA) | Yes: | Claims | 1-7,15-28,33,35-38               |
|                               | No:  | Claims |                                  |

2. Citations and explanations  
**see separate sheet**

**SECTION III:**

Claims 8 to 14 and 34 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

**SECTION IV:**

The separate inventions are:

1. Claims 1 to 28 and 33 to 38:

Agents of formulae (I), (II-1) or (II-2), pharmaceutical compositions containing them and use thereof for treating dementia or amnesia.

2. Claims 29 to 32:

A screening method for agents with anti-dementia or anti-amnesia properties which comprises using a somatostatin releasing action as an index.

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) because the screening method may concern other compounds than those claimed in the first invention.

**SECTION V:**

- 1) The documents are numbered according to their sequence in the search report.
- 2) The examination has been carried out assuming that the priority is valid, so that the P-document D9 has not been taken into consideration.

- 3) Document D3 describes pharmaceutical compositions of N-(4-acetyl-1-piperazinyl)-4-fluorobenzamide hydrate, which falls under the general formula (I) of claim 3, as an antidementia drug (see in particular claims 6 and 7).

The same applies to the compounds of document D7 (see the claims and examples).

Therefore the subject-matter of claims 1 to 3, 6 to 10, 13 to 17, 20 to 24, 27, 28 and 33 to 38 is not novel (Article 33 (2) PCT).

- 4) The subject-matter of claims 4, 5, 11, 12, 18, 19, 25, 26 differs from the above cited prior art by the disclaimer "provided that when X is N, then J is...".

Therefore the subject-matter of these claims is novel.

However, a disclaimer may render the subject-matter of a claim novel, but not necessarily inventive. In view of the structural similarity of the compounds of documents D3 and D7 and the compounds of these claims, it was obvious for the person skilled in the art to use the structurally related compounds of these claims as antidementia drugs.

Therefore the subject-matter of these claims does not involve an inventive step (Article 33 (3) PCT).

However, if the applicant is able to show that the compounds of these claims show superior properties in relation to those of the cited documents, then an inventive step could be acknowledged.

- 5) For the assessment of the present claims 8 to 14 and 34 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

---

International application No. PCT/JP00/03334

and the use of such a compound for the manufacture of a medicament for a new medical treatment.

**SECTION VI:**

**Certain published documents (Rule 70.10)**

| Application No<br>Patent No | Publication date<br>(day/month/year) | Filing date<br>(day/month/year) | Priority date ( <i>valid claim</i> )<br>(day/month/year) |
|-----------------------------|--------------------------------------|---------------------------------|--|
| WO 00 42011                 | 20.7.00                              | 6.1.00                          | 14.1.99  |

## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

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|  |   |  |
|--|---|--|
| Applicant's or agent's file reference<br><b>09364</b>        | <b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. |  |
| International application No.<br><b>PCT/JP 00/ 03334</b>     | International filing date (day/month/year)<br><b>24/05/2000</b>   | (Earliest) Priority Date (day/month/year)<br><b>28/05/1999</b> |
| Applicant<br><b>FUJISAWA PHARMACEUTICAL CO., LTD. et al.</b> |   |  |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

**1. Basis of the report**

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/JP 00/03334

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-28, 33-38

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-28,33-38

Agents of formulae (I), (II-1) or (II-2), pharmaceutical compositions containing them, and use thereof for treating dementia or amnesia.

2. Claims: 29-32

A screening method for agents with anti-dementia or anti-amnesia properties comprises using a somatostatin releasing action as an index.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/JP 00/03334

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/495 A61K31/4468 A61K31/4545 G01N33/15

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal, BIOSIS, MEDLINE, EMBASE, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.  |
|------------|---|--|
| X          | <p>MATSUOKA, NOBUYA ET AL: "FK960 [N-(4-acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate], a novel potential antimentia drug, improves visual recognition memory in rhesus monkeys: comparison with physostigmine" J. PHARMACOL. EXP. THER. (1997), 280(3), 1201-1209, 1997, XP001015389 abstract</p> <p style="text-align: center;">---<br/>-/-</p> | <p>1-3,<br/>6-10,<br/>13-17,<br/>20-24,<br/>27,28,<br/>33-38</p> |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

7 September 2001

Date of mailing of the international search report

27.12.01

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

A. Jakobs

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/JP 00/03334

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.   |
|------------|--|---|
| X          | <p>GRAUL, A. ET AL: "FK-960. Cognition enhancer"<br/> DRUGS FUTURE (1997), 22(8), 830-832,<br/> 1997, XP001015394</p> <p>page 830, column 2, paragraph 3 -page 832,<br/> column 1, paragraph 2</p> <p>---</p>  | <p>1-3,<br/> 6-10,<br/> 13-17,<br/> 20-24,<br/> 27,28,<br/> 33-38</p> |
| X          | <p>WO 98 25914 A (BABA YUKIHISA ;MIMURA<br/> HISASHI (JP); FUJISAWA PHARMACEUTICAL CO<br/> (JP) 18 June 1998 (1998-06-18)</p> <p>page 1, line 1-22; claims 1,6,7</p> <p>---</p>  | <p>1-3,<br/> 6-10,<br/> 13-17,<br/> 20-24,<br/> 27,28,<br/> 33-38</p> |
| X          | <p>DATABASE BIOSIS [Online]<br/> BIOSCIENCES INFORMATION SERVICE,<br/> PHILADELPHIA, PA, US;<br/> 1 June 1998 (1998-06-01)<br/> MATSUOKA NOBUYA ET AL: "FK960, a novel<br/> potential anti-dementia drug, augments<br/> long-term potentiation in mossy fiber-CA3<br/> pathway of guinea-pig hippocampal slices."<br/> Database accession no. PREV199800350951<br/> XP002176821<br/> abstract<br/> &amp; BRAIN RESEARCH,<br/> vol. 794, no. 2, 1 June 1998 (1998-06-01),<br/> pages 248-254,<br/> ISSN: 0006-8993</p> <p>---</p> | <p>1-3,<br/> 6-10,<br/> 13-17,<br/> 20-24,<br/> 27,28,<br/> 33-38</p> |
| E          | <p>WO 00 42011 A (AOKI SATOSHI ;YAMADA AKIRA<br/> (JP); FUJISAWA PHARMACEUTICAL CO (JP))<br/> 20 July 2000 (2000-07-20)<br/> the whole document</p> <p>---</p>   | <p>1-28,<br/> 33-38</p>   |
| X          | <p>WO 98 35951 A (OHNE KAZUHIKO ;SHIMA ICHIRO<br/> (JP); FUJISAWA PHARMACEUTICAL CO (JP))<br/> 20 August 1998 (1998-08-20)</p> <p>page 1, line 16 -page 4, line 13<br/> page 24, line 2-13<br/> claims 1-6; examples 1-27</p> <p>---</p> <p>---<br/> -/--</p>  | <p>1-3,<br/> 6-10,<br/> 13-17,<br/> 20-24,<br/> 27,28,<br/> 33-38</p> |

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/JP 00/03334

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.  |
|------------|---|--|
| X          | <p>EP 0 436 734 A (FUJISAWA PHARMACEUTICAL CO) 17 July 1991 (1991-07-17)</p> <p>abstract<br/>page 2, line 14-27; claims 1,3-7; examples 1-6</p> <p style="text-align: center;">---</p>  | <p>1-3,<br/>6-10,<br/>13-17,<br/>20-24,<br/>27,28,<br/>33-38</p> |
| X          | <p>WO 97 36871 A (WHITTLE PETER JOHN ;LOWE JOHN ADAMS III (US); PFIZER (US)) 9 October 1997 (1997-10-09)</p> <p>abstract<br/>page 11, line 3 -page 13, line 2; claims 7,8; examples 3,10-13,25,42,81</p> <p style="text-align: center;">---</p>   | <p>1-3,<br/>6-10,<br/>13-17,<br/>20-24,<br/>27,28,<br/>33-38</p> |
| X,P        | <p>DATABASE WPI<br/>Section Ch, Week 200004<br/>Derwent Publications Ltd., London, GB;<br/>Class B02, AN 2000-052797<br/>XP002176822<br/>&amp; WO 99 55674 A (DAINIPPON PHARM CO LTD), 4 November 1999 (1999-11-04)<br/>table 7<br/>abstract</p> <p style="text-align: center;">-----</p> | <p>1,2,4-9,<br/>11-16,<br/>18-23,<br/>25-28,<br/>33-38</p>       |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/JP 00/03334

| Patent document<br>cited in search report |   | Publication<br>date | Patent family<br>member(s)  | Publication<br>date  |
|---|---|---------------------|---|--|
| WO 9825914                                | A | 18-06-1998          | AT 205198 T<br>AU 722095 B2<br>AU 5136898 A<br>CN 1245495 A ,B<br>DE 69706562 D1<br>EP 0944612 A1<br>ES 2160371 T3<br>WO 9825914 A1<br>JP 2000514460 T<br>TW 422844 B<br>US 6147079 A   | 15-09-2001<br>20-07-2000<br>03-07-1998<br>23-02-2000<br>11-10-2001<br>29-09-1999<br>01-11-2001<br>18-06-1998<br>31-10-2000<br>21-02-2001<br>14-11-2000   |
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| EP 0436734                                | A | 17-07-1991          | DE 69022965 D1<br>DE 69022965 T2<br>DK 436734 T3<br>EP 0436734 A1<br>HK 64196 A<br>WO 9101979 A1<br>JP 2531304 B2<br>US 5250528 A   | 16-11-1995<br>04-04-1996<br>20-11-1995<br>17-07-1991<br>19-04-1996<br>21-02-1991<br>04-09-1996<br>05-10-1993   |
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| WO 9955674                                | A | 04-11-1999          | AU 3171699 A<br>BR 9909991 A<br>CN 1298391 T<br>EP 1076055 A1<br>HU 0101511 A2  | 16-11-1999<br>26-12-2000<br>06-06-2001<br>14-02-2001<br>28-09-2001   |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/JP 00/03334

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|---|---------------------|----------------------------|---------------------|
| WO 9955674                                | A                   | WO 9955674 A1              | 04-11-1999          |
|   |                     | NO 20005430 A              | 20-12-2000          |
|   |                     | PL 343726 A1               | 10-09-2001          |
|   |                     | US 6294555 B1              | 25-09-2001          |
|   |                     | JP 2000080081 A            | 21-03-2000          |
| -----                                     |                     |                            |                     |

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



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7 December 2000 (07.12.2000)

PCT

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**WO 00/72834 A2**

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(21) International Application Number: PCT/JP00/03334

(22) International Filing Date: 24 May 2000 (24.05.2000)

(25) Filing Language: English

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(30) Priority Data:  
09/321,745 28 May 1999 (28.05.1999) US

(71) Applicant (for all designated States except US): FUJISAWA PHARMACEUTICAL CO., LTD. [JP/JP]; 4-7, Doshomachi 3-chome, Chuo-ku, Osaka-shi, Osaka 541-8514 (JP).

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(74) Agent: TAKASHIMA, Hajime; Yuki Bldg., 3-9, Hiranomachi 3-chome, Chuo-ku, Osaka-shi, Osaka 541-0046 (JP).

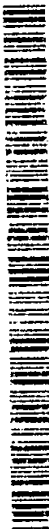
(81) Designated States (national): BR, CA, CN, JP, KR, US.

(84) Designated States (regional): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:

— Without international search report and to be republished upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 00/72834 A2

(54) Title: AGENT FOR EXPRESSION OF LONG-TERM POTENTIATION OF SYNAPTIC TRANSMISSION COMPRISING COMPOUND HAVING BRAIN SOMATOSTATIN ACTIVATION PROPERTY

(57) Abstract: The present invention relates to an agent for the expression of long-term potentiation of synaptic transmission, which contains a compound having a brain somatostatin activation property as an active ingredient and to a screening method of an agent for the expression of long-term potentiation of synaptic transmission, which uses a somatostatin releasing property as an index. The present invention is useful for the prophylaxis and/or treatment of cerebral diseases of dementia, amnesia, manic-depressive psychosis, schizophrenia, Parkinson's disease, psychosomatic disease and the like.



### DESCRIPTION

**AGENT FOR EXPRESSION OF LONG-TERM  
POTENTIATION OF SYNAPTIC TRANSMISSION COMPRISING  
COMPOUND HAVING BRAIN SOMATOSTATIN ACTIVATION PROPERTY**

**5** **Technical Field**

The present invention relates to an agent for the expression of long-term potentiation of synaptic transmission, an anti-dementia agent and an anti-amnesia agent, all of which containing a compound having a brain somatostatin activation property. More particularly, the present invention relates to an agent for the expression of long-term potentiation of synaptic transmission, an anti-dementia agent and an anti-amnesia agent, all of which containing a compound exerting a brain somatostatin release promoting action through suppression of the negative feedback mechanism of brain somatostatin release. The present invention moreover relates to a method for expressing long-term potentiation of synaptic transmission, a method for the treatment and/or prophylaxis of dementia and amnesia, and a screening method of these drugs using a somatostatin releasing property as an index.

## Background Art

20 The hippocampal function is said to be responsible for learning and memory. When an input neuron of the hippocampus is stimulated for a short time at high frequency, the efficiency of synaptic transmission continues to increase for a long time thereafter. This phenomenon is called long-term potentiation (hereinafter also referred to as LTP) 25 of synaptic transmission, and has been recognized as a cellular model of learning and memory (T. V. P. Bliss and G. L. Collingridge, Nature vol. 361, p. 31, 1993). There is a demand for further elucidation of the mechanism of the LTP and the relation thereof with learning and memory. Also, a search for a compound having a property of long-term 30 potentiation of synaptic transmission has been desired.

Somatostatin has been known for quite a long time as a hypothalamic hormone capable of suppressing the secretion of somatotropin from the pituitary gland. It has been recently found that it is also present in the cerebral cortex and the hippocampus, that are important cerebral sites for memory and learning, at high concentrations, playing an important role in memory and learning as a neuromodulator.

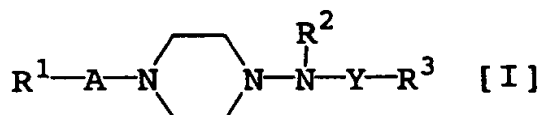
## Disclosure of the Invention

As a result of the intensive studies of the present inventors,

it has been newly found that a compound having a brain somatostatin release promotion property expresses long-term potentiation of synaptic transmission. Based on this new finding, the inventors have found that administration of a compound having a brain somatostatin release promoting property leads to the prophylaxis and/or treatment of cerebral diseases such as dementia, amnesia, manic-depressive psychosis, schizophrenia, Parkinson's disease, psychosomatic disease, and the like, which resulted in the completion of the present invention.

Accordingly, the present invention provides the following.

- (1) An agent for expression of long-term potentiation of synaptic transmission, which comprises a compound having a brain somatostatin activation property as an active ingredient;  
 a method for expressing long-term potentiation of synaptic transmission, comprising administering an effective amount of a compound having a brain somatostatin activation property;  
 use of a compound having a brain somatostatin activation property for the production of an agent for the expression of long-term potentiation of synaptic transmission; and  
 a pharmaceutical composition for expression of long-term potentiation of synaptic transmission, which comprises a compound having a brain somatostatin activation property, and a pharmaceutically acceptable carrier or excipient.
- (2) The agent, the method, the use and the pharmaceutical composition of (1), wherein the compound exerts an action to promote a release of brain somatostatin through suppression of a negative feedback mechanism of brain somatostatin release.
- (3) The agent, the method, the use and the pharmaceutical composition of (1) or (2), wherein the compound has the following formula [I]:

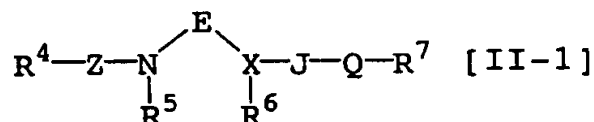


30

wherein

- $R^1$  is lower alkyl, aryl, ar(lower)alkoxy or heterocyclic group, each of which may be substituted with halogen,  
 $R^2$  is hydrogen atom or lower alkyl,  
 35  $R^3$  is cyclo(lower)alkyl, aryl or ar(lower)alkyl, each of which

- may be substituted with halogen,  
 A is -CO-, -SO<sub>2</sub>- or lower alkylene, and  
 Y is -CO-, -SO<sub>2</sub>- or -CONH-,  
 or pharmaceutically acceptable salts thereof.
- 5 (4) The agent, the method, the use and the pharmaceutical composition of (1) or (2), wherein the compound has the following formula [II-1]:



- wherein
- 10 R<sup>4</sup> is acyl,  
 R<sup>7</sup> is lower alkyl, lower alkoxy, lower alkylamino, lower alkenyl, lower alkenyloxy, lower alkenylamino, lower alkynyl, lower alkynyloxy, lower alkynylamino, cyclo(lower)alkyl, cyclo(lower)alkyloxy,  
 15 cyclo(lower)alkylamino, aryl, aryloxy, arylamino, a heterocyclic group or amino substituted with a heterocyclic group, each of which may be substituted with suitable substituent(s); or acyl;
- Z is a single bond, -CO- or -SO<sub>2</sub>-,  
 20 E is lower alkylene optionally substituted with suitable substituent(s),  
 X is CH or N,  
 J is a single bond, lower alkylene or



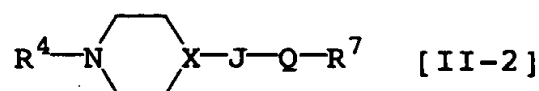
- 25 wherein R<sup>8</sup> is hydrogen, lower alkyl, substituted-lower alkyl, an N-protective group, aryl, acyl or a heterocyclic group,  
 Q is -CH<sub>2</sub>-, -CO-, -SO<sub>2</sub>- or -N=CH-, and  
 R<sup>5</sup> and R<sup>6</sup> are each hydrogen or lower alkyl, or are taken together  
 30 to form lower alkylene optionally condensed with a cyclic hydrocarbon or a heterocyclic ring,  
 provided that when X is N,

then 1) J is a single bond, and Q is  $-\text{CH}_2-$ ,  $-\text{CO}-$  or  $-\text{SO}_2-$ , or

2) J is lower alkylene,

or pharmaceutically acceptable salts thereof.

(5) The agent, the method, the use and the pharmaceutical composition  
5 of (1) or (2), wherein the compound has the following formula [II-2]:



wherein

$\text{R}^4$  is acyl,  
10  $\text{R}^7$  is aryl, aryloxy or arylamino, the aryl moiety of all of which may be substituted with halogen; pyridyl; or pyridylamino;  
 $\text{X}$  is CH or N,  
 $\text{J}$  is a single bond, lower alkylene or

15



wherein  $\text{R}^8$  is hydrogen, lower alkyl or an N-protective group,  
 $\text{Q}$  is  $-\text{CH}_2-$ ,  $-\text{CO}-$  or  $-\text{SO}_2-$ ,  
provided that when X is N, then J is a single bond or lower alkylene,  
20 or pharmaceutically acceptable salts thereof.

(6) The agent, the method and the pharmaceutical composition of any of (1) to (5), which is for the prophylaxis and/or treatment of cerebral diseases; and the use according to any of (1) to (5), which is for the production of an agent for the prophylaxis and/or treatment of  
25 cerebral diseases.

(7) The agent, the method and the pharmaceutical composition of (6), which is for the prophylaxis and/or treatment of dementia or amnesia; and the use according to (6), which is for the production of an agent for the prophylaxis and/or treatment of dementia or amnesia.

30 (8) A method for screening an agent for expression of long-term potentiation of synaptic transmission, which comprises using a somatostatin releasing action as an index.

(9) The screening method of (8), which is a screening method of an

anti-dementia agent or anti-amnesia agent.

(10) A method for screening an agent for expression of long-term potentiation of synaptic transmission, which comprises stimulating hippocampal slices, bringing a hippocampal slice into contact with a test compound, measuring an amount of somatostatin released from the hippocampal slice and/or a release time thereof, measuring an amount of somatostatin released from a hippocampal slice and/or a release time thereof in the absence of a contact with the test compound, and comparing the amounts and/or the times to calculate the amount of somatostatin released from the hippocampal slice and/or the release time thereof caused by the contact with the test compound.

(11) The screening method according to (10), which is a screening method of an anti-dementia agent or anti-amnesia agent.

(12) The agent, the method, the use and the pharmaceutical composition according to (1), wherein the compound having the brain somatostatin activation property is obtained by the screening method of any of (8) to (11).

(13) A commercial package comprising the pharmaceutical composition for expression of long-term potentiation of synaptic transmission of any of (1) - (7), (12) and a written matter associated therewith, wherein the written matter states that the pharmaceutical composition can or should be used for expression of long-term potentiation of synaptic transmission.

(14) A compound selected by the screening method described in any of (8) to (11).

#### Brief Description of the Drawing

Fig. 1 is a bar graph showing the action of compound 1 to be mentioned later, i.e., N-(4-acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate, on somatostatin release from rat hippocampal slice upon stimulation with 50mM K<sup>+</sup>, wherein the axis of ordinate is somatostatin release (%) and each value is mean  $\pm$  S.E.M (n=10 - 11). The symbol \* means that Dunnett's multiple comparison test showed a significant difference by P<0.05 of the group containing various concentrations of compound 1 as compared to the control group.

Fig. 2 is a bar graph showing the action of compound 1 on hippocampus long-term potentiation phenomenon, wherein the axis of ordinate shows the magnitude of LTP by the integral (%·min) of potential variation (%) from 12 minutes to 62 minutes after tetanic stimulation. The symbol

\* means that one-way analysis of variance and Dunnett's multiple comparison test showed a significant difference by  $P < 0.05$  of the group containing various concentrations of compound 1 as compared to the control group. The symbol \*\* means that one-way analysis of variance and Dunnett's multiple comparison test showed a significant difference by  $P < 0.01$  of the group containing various concentrations of compound 1 as compared to the control group.

Fig. 3 is a bar graph showing the dose-response dependency of compound 1 with regard to voltage-dependent calcium channel, wherein the axis of ordinate shows variation (%) of the maximal value of the membrane potential dependent calcium current to the value before the addition of compound 1, wherein each value is  $\text{mean} \pm \text{S.E.M}$  ( $n=7$ ). The symbol \* means that Dunnett's multiple comparison test showed a significant difference by  $P < 0.05$  of the group containing various concentrations of compound 1 as compared to the control group. The symbol \*\* means that Dunnett's multiple comparison test showed a significant difference by  $P < 0.01$  of the group containing various concentrations of compound 1 as compared to the control group.

Fig. 4 is a bar graph showing the action of somatostatin and compound 1 on the membrane potential dependent calcium current, wherein the axis of ordinate shows variation (%) of the maximal value of the membrane potential dependent calcium current upon addition of somatostatin alone or both compound 1 and somatostatin, to the value before addition of somatostatin ( $10^{-7}$  M) alone or both compound 1 ( $10^{-7}$  M) and somatostatin ( $10^{-7}$  M) and each value is  $\text{mean} \pm \text{S.E.M}$ ., and the numerals in parentheses are the number of times of measurements. The symbol \*\* means that Dunnett's multiple comparison test showed a significant difference by  $P < 0.01$  of the group containing compound 1 and somatostatin as compared to the group containing somatostatin.

#### Detailed Description of the Invention

Respective definitions and specific examples thereof used in the present invention, as well as preferable embodiments thereof are explained in detail in the following.

#### Compound having brain somatostatin activation property

The activation property of brain somatostatin means, for example, an action to promote release of brain somatostatin, an action to increase biosynthesis of somatostatin within nerve cells, an action to activate somatostatin receptors, an action to potentiate expression of

somatostatin property, an action to potentiate somatostatin signal transduction and the like.

The compound to be used in the present invention is one capable of activating brain somatostatin based on at least one of the  
5   aforementioned properties. Particularly, a compound having a brain somatostatin release promoting property is preferably used, which is preferably a compound that shows a brain somatostatin release promoting property through suppression of the negative feedback mechanism of brain somatostatin release.

10       Examples of promotion of release of brain somatostatin include actions of, for example, suppression of a negative feedback mechanism of brain somatostatin release, release of suppression by somatostatin of voltage-dependent calcium channel present in neuron, promotion of the voltage-dependent calcium channel, modification of mutual  
15   intracellular action between G protein and calcium channel, phosphorylation of calcium channel, modification of  $K^+$  channel, influencing kinetic behavior of somatostatin-containing vesicle, and the like, whereby release of somatostatin is promoted.

The action to increase biosynthesis of somatostatin in the nerve  
20   cells may be, for example, an action to potentiate the expression of somatostatin mRNA, an action to promote protein synthesis from mRNA, or an action to promote cleaving out from the precursor of somatostatin, wherein these actions promote the release of brain somatostatin.

The brain somatostatin release promoting property is evaluated  
25   by the method to be mentioned later.

The action to activate somatostatin receptors may be, for example, the actions to stimulate somatostatin receptors, to suppress desensitization of somatostatin receptors, to suppress intracellular transfer of somatostatin receptors, to increase the number of  
30   somatostatin receptors present in the postsynaptic membrane and the like.

The action to potentiate the expression of somatostatin property may be, for example, an action to suppress the decomposition of somatostatin, an action to suppress the re-uptake of somatostatin and  
35   the like.

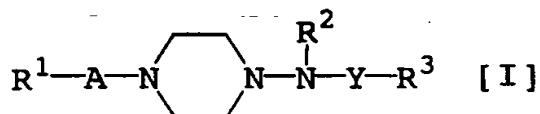
The action to potentiate the signal transmission of somatostatin is exemplified by an action to potentiate G protein, cAMP, protein kinase, protein phosphatase, transcription factor and the like, coupled

with a somatostatin receptor, wherein the action is directed to an intracellular signal transduction messenger and the like other than somatostatin receptor, thereby to potentiate the signal transduction of somatostatin.

- 5 The compound having a brain somatostatin activation property to be used in the present invention encompasses any compound having such an activation property. Preferable examples thereof include compounds of the following formulas:

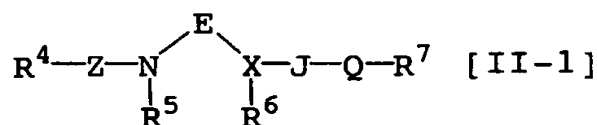
① formula [I]

10



wherein

- $\text{R}^1$  is lower alkyl, aryl, ar(lower)alkoxy or heterocyclic group, each of which may be substituted with halogen,  
 15  $\text{R}^2$  is hydrogen atom or lower alkyl,  
 $\text{R}^3$  is cyclo(lower)alkyl, aryl or ar(lower)alkyl, each of which may be substituted with halogen,  
 A is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or lower alkylene, and  
 Y is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or  $\text{---CONH---}$   
 20 (EP Publication No. 436734) (hereinafter also referred to as compound [I]), and pharmaceutically acceptable salts thereof and  
 ② formula [II-1]:

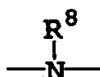


25 wherein

- $\text{R}^4$  is acyl,  
 $\text{R}^7$  is lower alkyl, lower alkoxy, lower alkylamino, lower alkenyl, lower alkenyloxy, lower alkenylamino, lower alkynyl, lower alkynyloxy, lower alkynylamino,  
 30 cyclo(lower)alkyl, cyclo(lower)alkyloxy, cyclo(lower)alkylamino, aryl, aryloxy, arylamino, a heterocyclic group or amino substituted with



- a heterocyclic group, each of which may be substituted with suitable substituent(s); or acyl;
- Z is a single bond, -CO- or -SO<sub>2</sub>-,
- E is lower alkylene optionally substituted with suitable substituent(s),
- X is CH or N,
- J is a single bond, lower alkylene or



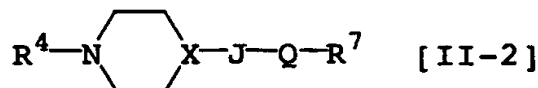
- wherein R<sup>8</sup> is hydrogen, lower alkyl, substituted-lower alkyl, an N-protective group, aryl, acyl or a heterocyclic group,
- Q is -CH<sub>2</sub>-, -CO-, -SO<sub>2</sub>- or -N=CH-, and
- R<sup>5</sup> and R<sup>6</sup> are each hydrogen or lower alkyl, or are taken together to form lower alkylene optionally condensed with a cyclic hydrocarbon or a heterocyclic ring,
- provided that when X is N,
- then 1) J is a single bond, and Q is -CH<sub>2</sub>-, -CO- or -SO<sub>2</sub>-, or
- 2) J is lower alkylene,
- (hereinafter also referred to as compound [II-1]) and pharmaceutically acceptable salts thereof.

Preferred compound [I] is one which has a lower alkyl, phenyl, naphthyl or thienyl for R<sup>1</sup>, hydrogen or lower alkyl for R<sup>2</sup>, phenyl which may be substituted with a halogen for R<sup>3</sup>, -CO- for A, and -CO- or -SO<sub>2</sub>- for Y.

- More preferred compound [I] is one which has a lower alkyl for R<sup>1</sup>, hydrogen for R<sup>2</sup>, phenyl which is substituted with a halogen for R<sup>3</sup>, -CO- for A, and -CO- for Y.

- Most preferred compound [I] is
- N-(4-acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate (compound 1) (International Publication No. WO98/25914).

When Z is a single bond, E is ethylene, and R<sup>5</sup> and R<sup>6</sup> are taken together to form ethylene, preferable compounds [II-1] can be represented by the following general formula [II-2]:



wherein

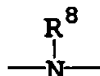
$R^4$  is acyl,

5  $R^7$  is aryl, aryloxy or arylamino, the aryl moiety of all of which may be substituted with halogen; pyridyl; or pyridylamino;

X is CH or N,

J is a single bond, lower alkylene or

10



wherein  $R^8$  is hydrogen, lower alkyl or an N-protective group,

Q is  $-CH_2-$ ,  $-CO-$  or  $-SO_2-$ ,

provided that when X is N, then J is a single bond or lower alkylene,  
15 (hereinafter also referred to as compound [II-2]) and pharmaceutically acceptable salts thereof.

Preferred compound [II-2] is one which has lower alkanoyl, esterified carboxy, substituted or unsubstituted aroyl, lower alkylsulfonyl, substituted or unsubstituted arylsulfonyl, or  
20 cyclo(lower)alkylcarbonyl for  $R^4$ , and aryl or arylamino, each aryl of which may be substituted with halogen for  $R^7$ , CH or N for X, a single bond, lower alkylene or



25 (wherein  $R^8$  is hydrogen, lower alkyl or an N-protective group) for J, and  $-CH_2-$ ,  $-CO-$  or  $-SO_2-$  for Q,

provided that when X is N, then J is a single bond or lower alkylene, and pharmaceutically acceptable salts thereof.

More preferred compound [II-2] is one which has  
30 lower alkanoyl, lower alkoxy carbonyl, aroyl, aroyl substituted with

halo(lower)alkoxy, lower alkylsulfonyl, arylsulfonyl, arylsulfonyl substituted with halogen, or cyclo(lower)alkylcarbonyl for R<sup>4</sup>, aryl or arylamino, each aryl of which may be substituted with halogen for R<sup>7</sup>, CH for X, a single bond or -NH- for J, and -CO- or -SO<sub>2</sub>- for Q, and pharmaceutically acceptable salts thereof.

Particularly more preferred compound [II-2] is one which has lower alkanoyl, lower alkoxycarbonyl, aroyl, aroyl substituted with halo(lower)alkoxy, lower alkylsulfonyl, arylsulfonyl, arylsulfonyl substituted with halogen, or cyclo(lower)alkylcarbonyl for R<sup>4</sup>, aryl or arylamino, each aryl of which may be substituted with halogen for R<sup>7</sup>, CH for X, -NH- for J, and -CO- for Q, and pharmaceutically acceptable salts thereof.

Most preferred compound [II-2] is one selected from the group consisting of

N-(1-acetylpiperidin-4-yl)-4-fluorobenzamide,  
 N-(1-acetylpiperidin-4-yl)-N'-(4-fluorophenyl)urea,  
 4-(4-fluorobenzoylamino)-1-methoxycarbonylpiperidine,  
 4-(4-fluorobenzoylamino)-1-(4-fluorophenylsulfonyl)piperidine,  
 4-(4-fluorobenzoylamino)-1-(4-trifluoromethoxybenzoyl)piperidine,  
 4-(4-fluorobenzoylamino)-1-methylsulfonylpiperidine,  
 N-(1-methoxycarbonylpiperidin-4-yl)-N'-(4-fluorophenyl)urea,  
 N-(1-(4-fluorophenylsulfonyl)piperidin-4-yl)-N'-(4-fluorophenyl)-  
 urea,  
 N-(1-benzoylpiperidin-4-yl)-4-fluorobenzamide,  
 N-(1-pivaloylpiperidin-4-yl)-4-fluorobenzamide and  
 N-(1-cyclopropylcarbonylpiperidin-4-yl)-4-fluorobenzamide.

In the above and subsequent description of the present specification, suitable examples of the various definitions to be included within the scope of the invention are explained in detail in the following.

The term "lower" is intended to mean a group having 1 to 6 carbon atom(s), unless otherwise provided.

The lower moiety in the terms "lower alkenyl", "lower alkenyloxy", "lower alkenylamino", "lower alkynyl", "lower alkynyloxy" and "lower alkynylamino" is intended to mean a group having 2 to 6 carbon atoms.

The lower moiety in the terms "cyclo(lower)alkyl", "cyclo(lower)alkyloxy" and "cyclo(lower)alkylamino" is intended to mean a group having 3 to 6 carbon atoms.

Suitable "lower alkyl" and lower alkyl moiety in the terms "substituted-lower alkyl", "ar(lower)alkyl", "halo(lower)alkyl", "lower alkylamino", "lower alkylsilyl", "lower alkylthio" and "lower alkylsulfonyl" may be a straight or branched C<sub>1</sub>-C<sub>6</sub> alkyl such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, ethylpropyl, hexyl or the like, in which preferable one is methyl.

Suitable "lower alkenyl" and lower alkenyl moiety in the terms "lower alkenyloxy" and "lower alkenylamino" may be a straight or branched C<sub>2</sub>-C<sub>6</sub> alkenyl such as ethenyl, propenyl, butenyl, pentenyl, hexenyl, isopropenyl, butadienyl, pentadienyl, hexadienyl or the like, in which preferable one is ethenyl, propenyl or butadienyl.

Suitable "lower alkynyl" and lower alkynyl moiety in the terms "lower alkynyloxy" and "lower alkynylamino" may be a straight or branched C<sub>2</sub>-C<sub>6</sub> alkynyl such as ethynyl, propargyl, butynyl or the like, in which preferable one is ethynyl.

Suitable "cyclo(lower)alkyl" and cyclo(lower)alkyl moiety in the terms "cyclo(lower)alkyloxy" and "cyclo(lower)alkylamino" may be cyclo(C<sub>3</sub>-C<sub>6</sub>)alkyl such as cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl, in which preferable one in the definitions of general formulas [II-1] and [II-2] is cyclopropyl.

Suitable "aryl" in the definitions of general formula [I] may be phenyl, naphthyl, tolyl, xylyl, mesityl, cumenyl, and the like, in which preferable one is phenyl or naphthyl.

Suitable "ar(lower)alkoxy" in the definitions of general formula [I] may be benzyloxy, phenethyloxy, phenylpropoxy, benzhydryloxy, trityloxy and the like.

Suitable "aryl" and aryl or ar moiety in the terms "ar(lower)alkoxy", "aryloxy", "arylamino", "arylsulfonyl", "aroxy" and "ar(lower)alkyl" in the definitions of general formulas [II-1] and [II-2] may be phenyl, naphthyl, phenyl substituted with lower alkyl [e.g. tolyl, xylyl, mesityl, cumenyl, di(tert-butyl)phenyl, etc.] and the like, in which preferable one is phenyl or tolyl.

Suitable "ar(lower)alkyl" may be benzyl, phenethyl, phenylpropyl, benzhydryl, trityl and the like, in which preferable one in the definitions of general formulas [II-1] and [II-2] is benzyl.

Suitable "lower alkylene" in the definitions of general formula [I] may be methylene, ethylene, propylene, pentamethylene, hexamethylene, and the like.

Suitable "lower alkylene" and lower alkylene moiety in the term "lower alkylenedioxy" in the definitions of general formulas [II-1] and [II-2] may be a straight or branched C<sub>1</sub>-C<sub>6</sub> alkylene such as methylene, ethylene, trimethylene, propylene, tetramethylene, pentamethylene, 5 hexamethylene, ethylethylene or the like, in which preferable one is methylene, ethylene or trimethylene.

Suitable "lower alkoxy" and lower alkoxy moiety in the terms "ar(lower)alkoxy" and "halo(lower)alkoxy" may be a straight or branched C<sub>1</sub>-C<sub>6</sub> alkoxy such as methoxy, ethoxy, propoxy, isopropoxy, methylpropoxy, 10 butoxy, isobutoxy, tert-butoxy, pentyloxy, hexyloxy or the like, in which preferable one is methoxy or tert-butoxy.

Suitable "heterocyclic group" in the definitions of general formula [I] may include saturated or unsaturated, monocyclic or polycyclic one containing at least one hetero atom such as nitrogen 15 atom, oxygen atom or sulfur atom. The preferred examples of thus defined "heterocyclic group" may be

- unsaturated, 3 to 8-membered, more preferably 5 or 6-membered heteromonocyclic group containing 1 to 4-nitrogen atom(s), for example, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyridyl N-oxide, 20 dihydropyridyl, tetrahydropyridyl, pyrimidyl, pyrazinyl, pyridazinyl, triazinyl, triazolyl, tetrazinyl, tetrazolyl, etc.;
- unsaturated, condensed heterocyclic group containing 1 to 5 nitrogen atom(s), for example, indolyl, isoindolyl, indoliziny, benzimidazolyl, quinolyl, isoquinolyl, indazolyl, benzotriazolyl, etc.;
- 25 unsaturated, 3 to 8-membered heteromonocyclic group containing 1 to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), for example, oxazolyl, isoxazolyl, oxadiazolyl etc.;
- saturated, 3 to 8-membered heteromonocyclic group containing 1 to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), for example, morpholino, 30 sydnonyl, etc.;
- unsaturated, condensed heterocyclic group containing 1 to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), for example, benzoxazolyl, benzoxadiazolyl, etc.;
- unsaturated, 3 to 8-membered heteromonocyclic group containing 1 to 35 2 sulfur atom(s) and 1 to 3 nitrogen atom(s), for example, thiazolyl, isothiazolyl, thiadiazolyl etc.;
- unsaturated, 3 to 8-membered heteromonocyclic group containing 1 to 2 sulfur atom(s), for example, thienyl, etc.;

unsaturated, condensed heterocyclic group containing 1 to 2 sulfur atom(s) and 1 to 3 nitrogen atom(s), for example, benzothiazolyl, benzothiadiaazolyl, etc.;

5 unsaturated, 3 to 8-membered heteromonocyclic group containing an oxygen atom, for example, furyl, etc.;

unsaturated, condensed heterocyclic group containing 1 to 2 sulfur atom(s), for example, benzothienyl, etc.;

unsaturated, condensed heterocyclic group containing 1 to 2 oxygen atom(s), for example, benzofuranyl, etc.; or the like.

10 The above-mentioned "lower alkyl", "aryl", "ar(lower)alkoxy", "heterocyclic group", "cyclo(lower)alkyl" and "ar(lower)alkyl" in the definitions of general formula [I] may be substituted with halogen [e.g. fluorine, chlorine, bromine and iodine].

Suitable "halogen" and halomoiety in the term "halo(lower)alkyl" may be fluorine, chlorine, bromine and iodine, in which preferable one is fluorine, chlorine or iodine.

15 Suitable "halo(lower)alkyl" may be lower alkyl substituted with one or more halogens such as chloromethyl, dichloromethyl, fluoromethyl, difluoromethyl, trifluoromethyl, pentachloroethyl or the like, in which preferable one is trifluoromethyl.

Suitable "halo(lower)alkoxy" may be lower alkoxy substituted with one or more halogens such as chloromethoxy, dichloromethoxy, fluoromethoxy, difluoromethoxy, trifluoromethoxy, pentachloromethoxy or the like, in which preferable one is trifluoromethoxy.

25 Suitable "lower alkylamino" may be mono or di(lower)alkylamino such as methylamino, ethylamino, propylamino, isopropylamino, butylamino, tert-butylamino, isobutylamino, pentylamino, hexylamino, dimethylamino, diethylamino, dipropylamino, dibutylamino, diisopropylamino, dipentylamino, dihexylamino, N-methylethylamino or  
30 the like, in which preferable one is dimethylamino.

Suitable "lower alkylsilyl" may be mono, di, or tri(lower)alkylsilyl such as trimethylsilyl, dimethylsilyl, triethylsilyl or the like, in which preferable one is trimethylsilyl.

Suitable "lower alkylenedioxy" may be methylenedioxy, ethylenedioxy and the like, in which preferable one is methylenedioxy.

35 Suitable "heterocyclic group" in the definitions of general formulas [II-1] and [II-2] may be one containing at least one hetero atom selected from nitrogen, sulfur and oxygen atom, and may include

saturated or unsaturated, monocyclic or polycyclic heterocyclic group, and preferable heterocyclic group may be N-containing heterocyclic group such as

- 5   unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 4 nitrogen atom(s), for example, pyrrolyl, pyrrolinyl, imidazolyl, pyrazolyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazolyl [e.g. 4H-1,2,4-triazolyl, 1H-1,2,3-triazolyl, 2H-1,2,3-triazolyl, etc.], tetrazolyl [e.g. 1H-tetrazolyl, 2H-tetrazolyl, etc.], etc.;
- 10   saturated 3 to 7-membered heteromonocyclic group containing 1 to 4 nitrogen atom(s), [e.g. pyrrolidinyl, imidazolidinyl, piperidyl, piperazinyl, homopiperazinyl, etc.];
- 15   unsaturated condensed heterocyclic group containing 1 to 5 nitrogen atom(s), for example, indolyl, isoindolyl, indolizinyl, benzimidazolyl, quinolyl, isoquinolyl, imidazopyridyl, indazolyl, benzotriazolyl, tetrazolopyridazinyl [e.g. tetrazolo[1,5-b]pyridazinyl, etc.], quinoxalinyl, etc.;
- 20   unsaturated 3 to 6-membered heteromonocyclic group containing an oxygen atom, for example, pyranyl, furyl, etc.;
- 25   saturated 3 to 6-membered heteromonocyclic group containing an oxygen atom, for example, 1H-tetrahydropyranyl, tetrahydrofuranlyl, etc.;
- 30   unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 2 sulfur atom(s), for example, thienyl, etc.;
- 35   unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), for example, oxazolyl, isoxazolyl, oxadiazolyl [e.g. 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,5-oxadiazolyl, etc.], oxazolinyl [e.g. 2-oxazolinyl, etc.], etc.;
- 40   saturated 3 to 6-membered heteromonocyclic group containing 1 to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s) [e.g. morpholinyl, etc.];
- 45   unsaturated condensed heterocyclic group containing 1 to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s) [e.g. benzofurazanyl, benzoxazolyl, benzoxadiazolyl, etc.];
- 50   unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 2 sulfur atom(s) and 1 to 3 nitrogen atom(s), for example, thiazolyl, thiadiazolyl [e.g. 1,2,4-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl, etc.], etc.;
- 55   saturated 3 to 6-membered heteromonocyclic group containing 1 to 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) [e.g. thiazolidinyl, etc.];
- 60   unsaturated condensed heterocyclic group containing 1 to 2 sulfur

atom(s) and 1 to 3 nitrogen atom(s) [e.g. benzothiazolyl, benzothiadiazolyl, etc.];  
unsaturated condensed heterocyclic group containing 1 to 2 oxygen atom(s) [e.g. benzofuranyl, benzodioxolyl, chromanyl, etc.] and the like.

Said "heterocyclic group" may be substituted with lower alkyl as exemplified above, in which preferable one is thienyl, pyridyl, methylpyridyl, quinolyl, indolyl, quinoxaliny, benzofuranyl or tetramethylchromanyl, and more preferable one is pyridyl.

Suitable "acyl" may be carboxy; esterified carboxy; carbamoyl substituted with lower alkyl, aryl, ar(lower)alkyl, arylsulfonyl, lower alkylsulfonyl or a heterocyclic group; substituted or unsubstituted arylsulfonyl; lower alkylsulfonyl; cyclo(lower)alkylcarbonyl; lower alkanoyl; substituted or unsubstituted aroyl; a heterocycliccarbonyl and the like.

The esterified carboxy may be substituted or unsubstituted lower alkoxycarbonyl [e.g. methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl, tert-butoxycarbonyl, hexyloxycarbonyl, 2-iodoethoxycarbonyl, 2,2,2-trichloroethoxycarbonyl, etc.], substituted or unsubstituted aryloxycarbonyl [e.g. phenoxycarbonyl, 4-nitrophenoxycarbonyl, 2-naphthyloxycarbonyl, etc.], substituted or unsubstituted ar(lower)alkoxycarbonyl [e.g. benzyloxycarbonyl, phenethyloxycarbonyl, benzhydryloxycarbonyl, 4-nitrobenzyloxycarbonyl, etc.], and the like, in which preferable one is unsubstituted lower alkoxycarbonyl and more preferable one is methoxycarbonyl or tert-butoxycarbonyl.

The carbamoyl substituted with lower alkyl may be methylcarbamoyl, ethylcarbamoyl, propylcarbamoyl, dimethylcarbamoyl, diethylcarbamoyl, N-methyl-N-ethylcarbamoyl and the like.

The carbamoyl substituted with aryl may be phenylcarbamoyl, naphthylcarbamoyl, lower alkyl-substituted phenylcarbamoyl [e.g. tolylcarbamoyl, xylylcarbamoyl, etc.] and the like.

The carbamoyl substituted with ar(lower)alkyl may be benzylcarbamoyl, phenethylcarbamoyl, phenylpropylcarbamoyl and the like, in which preferable one is benzylcarbamoyl.

The carbamoyl substituted with arylsulfonyl may be phenylsulfonylcarbamoyl, tolylsulfonylcarbamoyl and the like.



The carbamoyl substituted with lower alkylsulfonyl may be methylsulfonylcarbamoyl, ethylsulfonylcarbamoyl and the like.

The carbamoyl substituted with a heterocyclic group may be one substituted with a heterocyclic group as mentioned above for the  
5 definitions of general formulas [II-1] and [II-2].

The lower alkanoyl may be formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, hexanoyl and the like, in which preferable one is acetyl or pivaloyl.

The substituted or unsubstituted aroyl may be benzoyl, naphthoyl,  
10 toluoyl, di(tert-butyl)benzoyl, halo(lower)alkoxybenzoyl [e.g. trifluoromethoxybenzoyl, etc.] and the like, in which preferable one is benzoyl or trifluoromethoxybenzoyl.

The substituted or unsubstituted arylsulfonyl may be phenylsulfonyl, tolylsulfonyl, halophenylsulfonyl [e.g.  
15 fluorophenylsulfonyl, etc.] and the like, in which preferable one is fluorophenylsulfonyl.

The lower alkylsulfonyl may be methylsulfonyl, ethylsulfonyl and the like, in which preferable one is methylsulfonyl.

The cyclo(lower)alkylcarbonyl may be cyclo(C<sub>3</sub>-C<sub>6</sub>)alkylcarbonyl  
20 such as cyclopropylcarbonyl, cyclobutylcarbonyl, cyclopentylcarbonyl or cyclohexylcarbonyl, in which preferable one is cyclopropylcarbonyl.

The heterocyclic moiety in the term "a heterocycliccarbonyl" may be one mentioned above as a heterocyclic group for the definitions of general formulas [II-1] and [II-2].

Suitable "N-protective group" may be common N-protective group  
25 such as substituted or unsubstituted lower alkanoyl [e.g. formyl, acetyl, propionyl, trifluoroacetyl, etc.], lower alkoxycarbonyl [e.g. tert-butoxycarbonyl, tert-amylloxycarbonyl, etc.], substituted or unsubstituted aralkyloxycarbonyl [e.g. benzyloxycarbonyl,  
30 p-nitrobenzyloxycarbonyl, etc.], 9-fluorenylmethoxycarbonyl, substituted or unsubstituted arenesulfonyl [e.g. benzenesulfonyl, tosyl, etc.], nitrophenylsulfonyl, aralkyl [e.g. trityl, benzyl, etc.] or the like, in which preferable one is lower alkoxycarbonyl and more preferable one is tert-butoxycarbonyl.

Suitable "cyclic hydrocarbon" may be a saturated or unsaturated  
35 cyclic hydrocarbon such as cyclopentane, cyclohexane, benzene, naphthalene, indan, indene or the like.

Suitable "substituted-lower alkyl" may be lower alkyl

substituted with halogen, aryl, acyl, lower alkoxy, aryloxy and the like, in which preferable one is benzyl.

Suitable "heterocyclic ring" may be one which is a heterocyclic group, as mentioned above for the definitions of general formulas  
5 [II-1] and [II-2], added by hydrogen.

Suitable lower alkylene condensed with a cyclic hydrocarbon may be lower alkylene condensed with benzene and the like, in which preferable one is ethylene condensed with benzene.

Suitable lower alkylene condensed with a heterocyclic ring may  
10 be lower alkylene condensed with pyridine and the like, in which preferable one is ethylene condensed with pyridine.

Preferred "acyl" for R<sup>4</sup> may be lower alkanoyl; lower alkoxycarbonyl; aroyl optionally substituted with halo(lower)alkoxy; arylsulfonyl optionally substituted with halogen; lower alkylsulfonyl;  
15 or cyclo(lower)alkylcarbonyl, in which more preferable one is acetyl, pivaloyl, methoxycarbonyl, tert-butoxycarbonyl, benzoyl, trifluoromethoxybenzoyl, fluorophenylsulfonyl, methylsulfonyl or cyclopropylcarbonyl.

Preferred "suitable substituent" as the substituent of lower  
20 alkyl, lower alkoxy, lower alkylamino, lower alkenyl, lower alkenyloxy, lower alkenylamino, lower alkynyl, lower alkynyloxy, lower alkynylamino, cyclo(lower)alkyl, cyclo(lower)alkyloxy, cyclo(lower)alkylamino, aryl, aryloxy, arylamino, a heterocyclic group or amino substituted with a heterocyclic group for R<sup>7</sup> may be halo(lower)alkyl,  
25 halo(lower)alkoxy, lower alkenyl, lower alkynyl, lower alkylamino, acylamino, acyl, lower alkylsilyl, lower alkoxy, aryl, lower alkylendioxy, acyloxy, hydroxy, nitro, amino, cyano, halogen, aryloxy, lower alkylthio and the like.

Preferred "aryl which may be substituted with suitable  
30 substituent(s)" for R<sup>7</sup> may be aryl optionally substituted with halogen, in which more preferable one is fluorophenyl.

Preferred "aryl amino which may be substituted with suitable substituent(s)" for R<sup>7</sup> may be aryl amino optionally substituted with halogen, in which preferable one is phenyl amino or fluorophenyl amino.

35 Preferred "aryloxy which may be substituted with suitable substituent(s)" for R<sup>7</sup> may be aryloxy optionally substituted with halogen, in which preferable one is fluorophenoxy.

Preferred "lower alkylene" for J may be methylene.

Preferred "lower alkyl" for R<sup>8</sup> in J may be methyl.

Preferred "N-protective group" for R<sup>8</sup> in J may be tert-butoxycarbonyl.

5 Preferred "suitable substituent" as the substituent of lower alkylene for E may be oxo, lower alkyl, hydroxy(lower)alkyl or acyl, in which more preferable one is oxo, dioxo, methyl, dimethyl, hydroxymethyl, or benzylcarbamoyle.

Preferred "lower alkylene" for E may be methylene, ethylene or trimethylene, and more preferable one is ethylene.

10 Preferred "lower alkyl" for R<sup>5</sup> and R<sup>6</sup> may be methyl.

Preferred "lower alkylene which R<sup>5</sup> and R<sup>6</sup> are taken together to form" may be ethylene or trimethylene.

Preferred "a cyclic hydrocarbon with which lower alkylene is condensed" may be benzene.

15 Another more preferred compound [II-2] is one having lower alkanoyl, lower alkoxy carbonyl, aroyl, aroyl substituted with halo(lower)alkoxy, lower alkylsulfonyl, arylsulfonyl, arylsulfonyl substituted with halogen, or cyclo(lower)alkylcarbonyl for R<sup>4</sup>, aryl or arylamino, each aryl of which may be substituted with halogen for R<sup>7</sup>, N for X, a single bond for J, and -CO- for Q.

20 Another most preferred compound [II-2] is one selected from the group consisting of

1-acetyl-4-(4-fluorophenylcarbamoyle)piperazine,  
1-tert-butoxycarbonyl-4-(4-fluorophenylcarbamoyle)piperazine,  
25 1-(4-fluorophenylcarbamoyle)-4-(4-trifluoromethoxybenzoyl)-piperazine and  
1-methoxycarbonyl-4-(4-fluorophenylcarbamoyle)piperazine.

Suitable pharmaceutically acceptable salts of the compounds of general formulas [I], [II-1] and [II-2] are conventional non-toxic  
30 salts and include acid addition salt such as an inorganic acid addition salt [e.g. hydrochloride, hydrobromide, sulfate, phosphate, etc.], an organic acid addition salt [e.g. formate, acetate, trifluoroacetate, maleate, tartrate, methanesulfonate, benzenesulfonate, toluenesulfonate, etc.], a salt with an amino acid [e.g. aspartic acid  
35 salt, glutamic acid salt, etc.], a metal salt such as an alkali metal salt [e.g. sodium salt, potassium salt, etc.] and alkaline earth metal salt [e.g. calcium salt, magnesium salt, etc.] and the like.

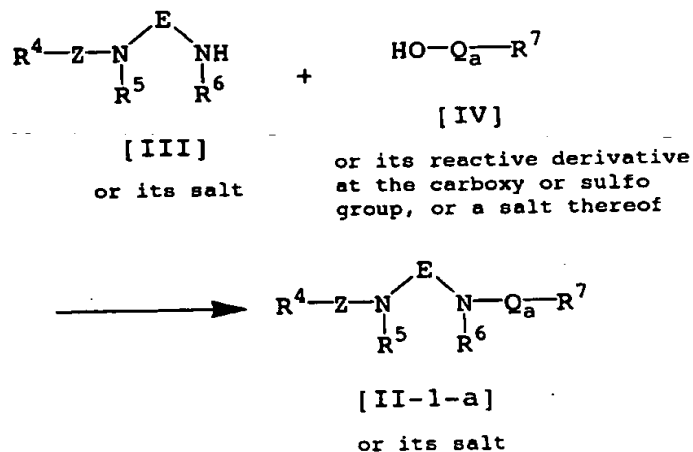
Compounds of the formula [I] and salts thereof can be prepared

according to the method disclosed in EP Publication No. 436734.

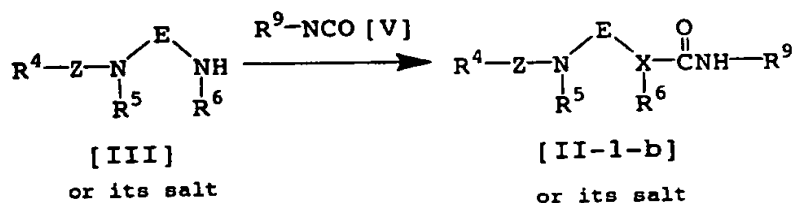
Compounds of the formula [II-1] including compounds [II-2] and salts thereof can be prepared by the processes as illustrated in the following reaction schemes.

5

Process 1

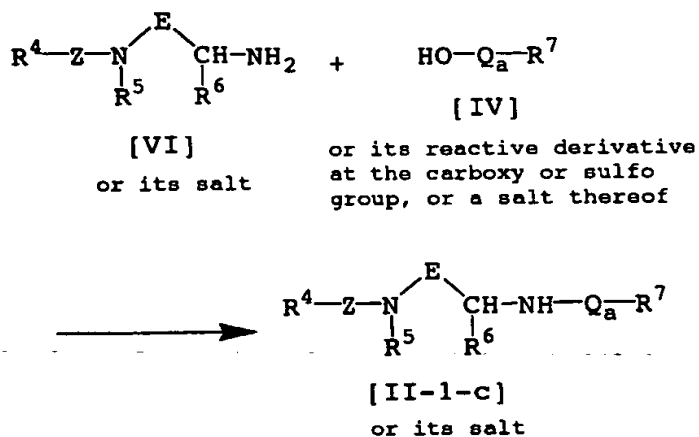


10 Process 2



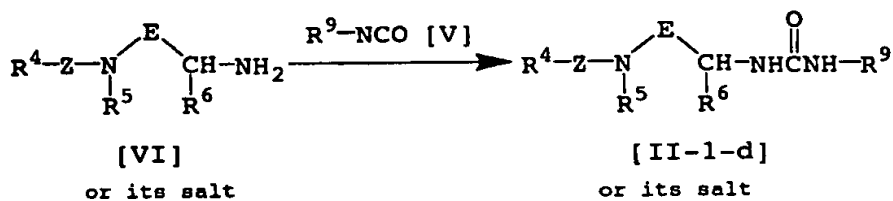
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### Process 3

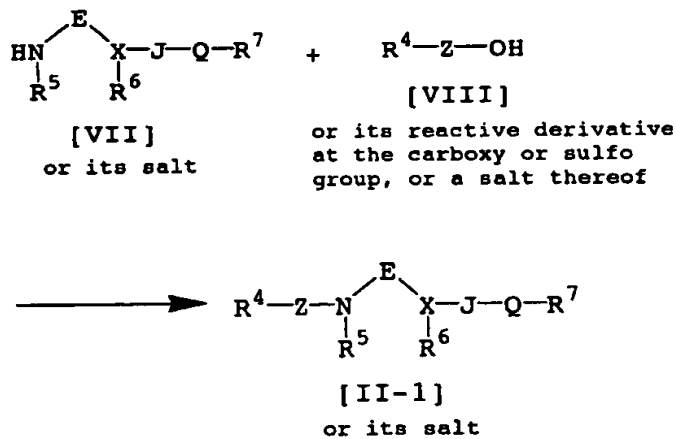


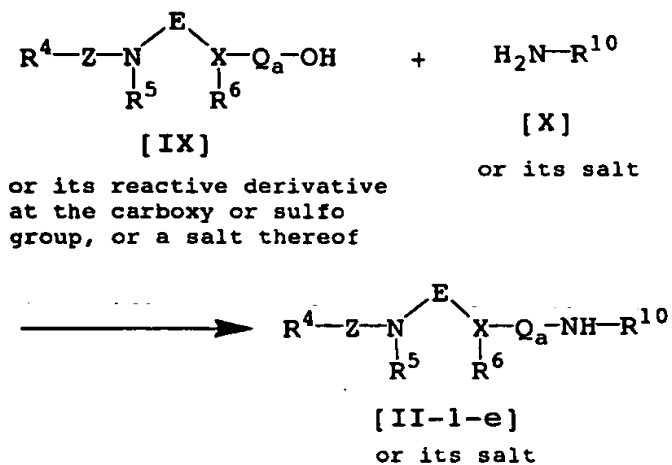
### Process 4

5

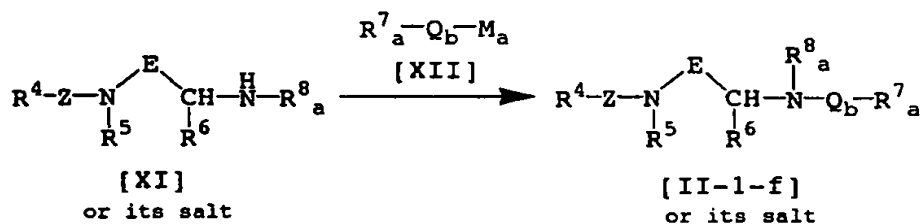


### Process 5

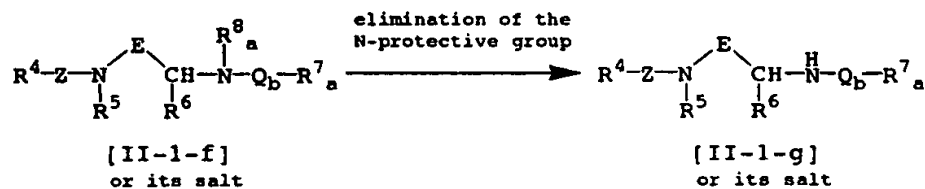


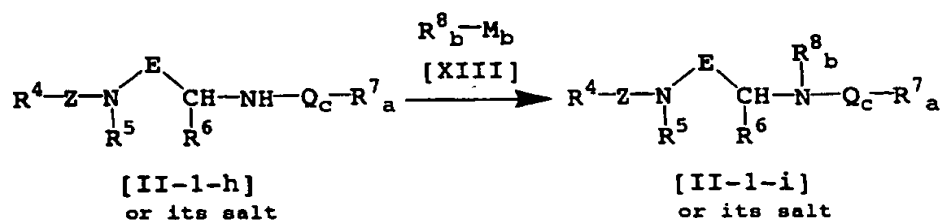
Process 6

5

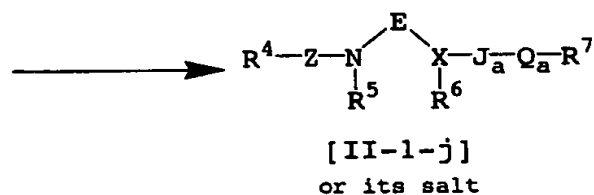
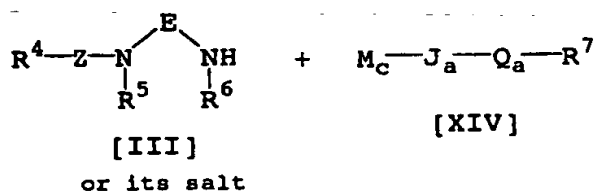
Process 7Process 8

10



Process 9Process 10

5



- 10 wherein  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $Z$ ,  $E$ ,  $Q$ ,  $X$  and  $J$  are each as defined above,  
 $Q_a$  is  $-CO-$  or  $-SO_2-$ ,  
 $R^9$  is aryl which may be substituted with suitable  
substituent(s), or pyridyl,  
 $R^{10}$  is lower alkyl, lower alkenyl, lower alkynyl,  
15 cyclo(lower)alkyl, aryl or a heterocyclic group, each of  
which may be substituted with suitable substituent(s),  
 $R^8_a$  is an N-protective group,  
 $R^7_a$  is lower alkyl, lower alkenyl, lower alkynyl,  
cyclo(lower)alkyl, aryl or a heterocyclic group, each of  
20 which may be substituted with suitable substituent(s),  
 $Q_b$  is  $-CH_2-$ ,  $-CO-$ , or  $-SO_2-$ ,

- $M_a$  is an acid residue,  
 $Q_c$  is  $-CO-$ ,  
 $R_b^s$  is lower alkyl,  
 $M_b$  is an acid residue,  
 5  $M_c$  is an acid residue, and  
 $J_a$  is lower alkylene.

Suitable "acid residue" may be halogen [e.g. fluoro, chloro, bromo, iodo], arenesulfonyloxy [e.g. benzenesulfonyloxy, tosyloxy, etc.], alkanesulfonyloxy [e.g. mesyloxy, ethansulfonyloxy, etc.], and the like, in which preferable one is halogen.

The processes for preparing the compounds [II-1] including compounds [II-2] are explained in detail in the following.

#### Process 1

The compound [II-1-a] or its salt can be prepared by reacting a compound [III] or its salt with a compound [IV] or its reactive derivative at the carboxy or sulfo group, or a salt thereof.

Suitable salts of the compounds [II-1-a] and [III] may be the same as those exemplified for the compound [II-1].

Suitable salts of the compound [IV] and its reactive derivative at the carboxy or sulfo group may be metal salt or alkaline earth metal salt as exemplified for the compound [II-1].

Suitable reactive derivative at the carboxy or sulfo group of the compound [IV] may include an ester, an acid halide, an acid anhydride and the like. The suitable examples of the reactive derivatives may be an acid halide [e.g. acid chloride, acid bromide, etc.]; a symmetrical acid anhydride; a mixed acid anhydride with an acid such as aliphatic carboxylic acid [e.g. acetic acid, pivalic acid, etc.], substituted phosphoric acid [e.g. dialkylphosphoric acid, diphenylphosphoric acid, etc.]; an ester such as substituted or unsubstituted lower alkyl ester [e.g. methyl ester, ethyl ester, propyl ester, hexyl ester, trichloromethyl ester, etc.], substituted or unsubstituted ar(lower)alkyl ester [e.g. benzyl ester, benzhydryl ester, p-chlorobenzyl ester, etc.], substituted or unsubstituted aryl ester [e.g. phenyl ester, tolyl ester, 4-nitrophenyl ester, 2,4-dinitrophenyl ester, pentachlorophenyl ester, naphthyl ester, etc.], or an ester with N,N-dimethylhydroxylamine, N-hydroxysuccinimide, N-hydroxyphthalimide, 1-hydroxybenzotriazole, 1-hydroxy-6-chloro-1H-benzotriazole, or the like. These reactive



derivatives can be optionally selected according to the kind of the compound [IV] to be used.

The reaction is usually carried out in a conventional solvent such as water, acetone, dioxane, chloroform, methylene chloride, ethylene dichloride, tetrahydrofuran, acetonitrile, ethyl acetate, N,N-dimethylformamide, pyridine or any other organic solvent which does not adversely influence the reaction. Among these solvents, hydrophilic solvent may be used in a mixture with water.

The reaction is also preferably carried out in the presence of a conventional base such as triethylamine, diisopropylethylamine, pyridine, N,N-dimethylaminopyridine, etc., or a mixture thereof.

When the compound [IV] is used in a free acid form or its salt form in the reaction, the reaction is preferably carried out in the presence of a conventional condensing agent such as N,N'-dicyclohexylcarbodiimide, N-cyclohexyl-N'-morpholinoethylcarbodiimide, N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide, thionyl chloride, oxalyl chloride, lower alkoxy carbonyl halide [e.g. ethyl chloroformate, isobutyl chloroformate, etc.], 1-(p-chlorobenzenesulfonyloxy)-6-chloro-1H-benzotriazole, or the like.

The reaction temperature is not critical, and the reaction can be carried out under cooling to heating.

#### Process 2

The compound [II-1-b] or its salt can be prepared by reacting a compound [III] or its salt with a compound [V].

Suitable salts of the compound [II-1-b] and [III] may be the same as those exemplified for the compound [II-1].

This reaction is usually carried out in a solvent such as dioxane, tetrahydrofuran, benzene, toluene, chloroform, methylene chloride or any other organic solvent which does not adversely influence the reaction.

The reaction temperature is not critical, and the reaction is usually carried out under cooling to warming.

#### Process 3

The compound [II-1-c] or its salt can be prepared by reacting a compound [VI] or its salt with a compound [IV] or its reactive derivative at the carboxy or sulfo group, or a salt thereof.

Suitable salts of the compounds [II-1-c] and [VI] may be the same as those exemplified for the compound [II-1].

Suitable salts of the compound [VI] and its reactive derivative at the carboxy or sulfo group may be metal salt or alkaline earth metal salt as exemplified for the compound [II-1].

This reaction can be carried out in substantially the same manner as Process 1, and therefore the reaction mode and reaction condition [e.g. solvent, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 1.

#### 10 Process 4

The compound [II-1-d] or its salt can be prepared by reacting a compound [VI] or its salt with a compound [V].

Suitable salts of the compound [II-1-d] and [VI] may be the same as those exemplified for the compound [II-1].

15 This reaction can be carried out in substantially the same manner as Process 2, and therefore the reaction mode and reaction condition [e.g. solvent, reaction temperature, etc.] of this reaction are to be referred to those explained in Process 2.

#### Process 5

20 The compound [II-1] or its salt can be prepared by reacting a compound [VII] or its salt with a compound [VIII] or its reactive derivative at the carboxy or sulfo group, or a salt thereof.

Suitable salt of the compound [VII] may be acid addition salt as exemplified for the compound [II-1].

25 Suitable salts of the compound [VIII] and its reactive derivative at the carboxy or sulfo group may be metal salt or alkaline earth metal salt as exemplified for the compound [II-1].

This reaction can be carried out in substantially the same manner as Process 1, and therefore the reaction mode and reaction condition [e.g. solvent, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 1.

30

#### Process 6

35 The compound [II-1-e] or its salt can be prepared by reacting a compound [IX] or its reactive derivative at the carboxy group or sulfo group, or a salt thereof with a compound [X] or its salt.

Suitable salts of the compounds [II-1-e], [IX] and its reactive derivative at the carboxy or sulfo group may be the same as those exemplified for the compound [II-1].

Suitable salt of the compound [X] may be acid addition salt as exemplified for the compound [II-1].

This reaction can be carried out in substantially the same manner as Process 1, and therefore the reaction mode and reaction condition [e.g. solvent, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 1.

#### Process 7

The compound [II-1-f] can be prepared by reacting a compound [XI] or its salt with a compound [XII].

Suitable salts of the compounds [II-1-f] and [XI] may be the same as those exemplified for the compound [II-1].

The present reaction is preferably carried out in the presence of base such as an alkali metal [e.g. lithium, sodium, potassium, etc.], alkaline earth metal [e.g. calcium, etc.], alkali metal hydride [e.g. sodium hydride, etc.], alkaline earth metal hydride [e.g. calcium hydride, etc.], the hydroxide or carbonate or bicarbonate of an alkali metal or an alkaline earth metal [e.g. potassium bicarbonate, etc.] and the like.

This reaction is usually carried out in a solvent such as N,N-dimethylformamide, diethyl ether, tetrahydrofuran, dioxane, benzene, toluene, acetonitrile or any other solvent which does not adversely influence the reaction.

The reaction temperature is not critical, and the reaction is usually carried out under cooling to heating.

#### Process 8

The object compound [II-1-g] or its salt can be prepared by subjecting a compound [II-1-f] or its salt to elimination reaction of the N-protective group.

Suitable salts of the compounds [II-1-f] and [II-1-g] may be acid addition salts as exemplified for the compound [II-1].

This reaction is carried out in accordance with a conventional method such as hydrolysis, reduction or the like.

The hydrolysis is preferably carried out in the presence of a base or an acid including Lewis acid.

Suitable base may include an inorganic base and an organic base such as an alkali metal [e.g. sodium, potassium, etc.], an alkaline earth metal [e.g. magnesium, calcium, etc.], the hydroxide or carbonate or bicarbonate thereof, hydrazine, alkylamine [e.g. methylamine,

trimethylamine, triethylamine, etc.], picoline, 1,5-diazabicyclo[4.3.0]non-5-ene, 1,4-diazabicyclo[2.2.2]octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, or the like.

Suitable acid may include an organic acid [e.g. formic acid, acetic acid, propionic acid, trichloroacetic acid, trifluoroacetic acid, etc.], an inorganic acid [e.g. hydrochloric acid, hydrobromic acid, sulfuric acid, hydrogen chloride, hydrogen bromide, hydrogen fluoride, etc.] and an acid addition salt compound [e.g. pyridine hydrochloride, etc.].

The elimination using in trihaloacetic acid [e.g. trichloroacetic acid, trifluoroacetic acid, etc.] or the like is preferably carried out in the presence of cation trapping agents [e.g. anisole, phenol, etc.].

The reaction is usually carried out in a solvent such as water, an alcohol [e.g. methanol, ethanol, etc.], methylene chloride, chloroform, tetrachloromethane, dioxane, tetrahydrofuran, a mixture thereof or any other solvent which does not adversely influence the reaction. A liquid base or acid can be also used as the solvent. The reaction temperature is not critical and the reaction is usually carried out under cooling to heating.

The reduction method applicable for the elimination reaction may include chemical reduction and catalytic reduction.

Suitable reducing agents to be used in chemical reduction are a combination of metal [e.g. tin, zinc, iron, etc.] or metallic compound [e.g. chromium chloride, chromium acetate, etc.] and an organic or inorganic acid [e.g. formic acid, acetic acid, propionic acid, trifluoroacetic acid, p-toluenesulfonic acid, hydrochloric acid, hydrobromic acid, etc.].

Suitable catalysts to be used in catalytic reduction are conventional ones such as platinum catalysts [e.g. platinum plate, spongy platinum, platinum black, colloidal platinum, platinum oxide, platinum wire, etc.], palladium catalysts [e.g. spongy palladium, palladium black, palladium oxide, palladium on carbon, colloidal palladium, palladium on barium sulfate, palladium on barium carbonate, etc.], nickel catalysts [e.g. reduced nickel, nickel oxide, Raney nickel, etc.], cobalt catalysts [e.g. reduced cobalt, Raney cobalt, etc.], iron catalysts [e.g. reduced iron, Raney iron, etc.], copper catalysts [e.g. reduced copper, Raney copper, Ullmann copper, etc.] and the like.

In case that the N-protective group is benzyl, the reduction is preferably carried out in the presence of a combination of palladium catalysts [e.g. palladium black, palladium on carbon, etc.] and formic acid or its salt [e.g. ammonium formate, etc.].

- 5       The reduction is usually carried out in a conventional solvent which does not adversely influence the reaction such as water, methanol, ethanol, propanol, N,N-dimethylformamide, or a mixture thereof. Additionally, in case that the above-mentioned acids to be used in chemical reduction are in liquid, they can also be used as a solvent.
- 10      Further, a suitable solvent to be used in catalytic reduction may be the above-mentioned solvent, and other conventional solvent such as diethyl ether, dioxane, tetrahydrofuran, etc. or a mixture thereof.

The reaction temperature of this reduction is not critical and the reaction is usually carried out under cooling the heating.

15      Process 9

The compound [II-1-i] or its salt can be prepared by reacting a compound [II-1-h] or its salt with a compound [XIII].

Suitable salts of the compounds [II-1-h] and [II-1-i] may be the same as those exemplified for the compound [II-1].

- 20      This reaction can be carried out in substantially the same manner as Process 7, and therefore the reaction mode and reaction condition [e.g. solvent, reaction temperature, etc.] of this reaction are to be referred to those explained in Process 7.

Process 10

- 25      The compound [II-1-j] or its salt can be prepared by reacting a compound [III] or its salt with a compound [XIV].

Suitable salts of the compounds [II-1-j] and [III] may be the same as those exemplified for the compound [II-1].

- 30      This reaction can be carried out in substantially the same manner as Process 7, and therefore the reaction mode and reaction condition [e.g. solvent, reaction temperature, etc.] of this reaction are to be referred to those explained in Process 7.

- 35      The compounds obtained by the above processes can be isolated and purified by a conventional method such as pulverization, recrystallization, column chromatography, reprecipitation, or the like.

It is to be noted that the compounds [I], [II-1], [II-2] and the other compounds may include one or more stereoisomer(s) such as

optical isomer(s) or geometrical isomer(s) due to asymmetric carbon atom(s) and double bond(s), and all of such isomers and mixture thereof are included within the scope of this invention.

- 5 Additionally, it is to be noted that any solvate [e.g. enclosure compound (e.g. hydrate, etc.)] of the compound [I], [II-1], [II-2] and pharmaceutically acceptable salts thereof is also included within the scope of this invention.

- 10 With regard to the brain somatostatin release promoting property, the brain somatostatin release promoting property is said to be expressed and the compound is said to have a brain somatostatin activation property, when the release amount of somatostatin by depolarization stimulation is increased due to a pretreatment of a hippocampal slice with a certain compound, as compared to when the pretreatment with the compound is  
15 void.

- With regard to the property of increasing biosynthesis of somatostatin, the property of increasing biosynthesis of somatostatin in nerve cells is said to be expressed and the compound is said to have a brain somatostatin activation property, when a somatostatin  
20 content of the nerve cells is increased in the nerve cells due to a pretreatment with a certain compound, as compared to when the pretreatment with the compound is void.

- With regard to the somatostatin receptor activation property, the somatostatin receptor activation property is said to be expressed  
25 and the compound is said to have a brain somatostatin activation property, when binding of somatostatin labeled with a radioisotope (e.g., <sup>125</sup>I and the like) to a somatostatin receptor in the nerve cells decreases due to a pretreatment with a certain compound, as compared to when the pretreatment with the compound is void.

- 30 With regard to the potentiation property of the expression of somatostatin property, the potentiation property of the expression of somatostatin property is said to be expressed and the compound is said to have a brain somatostatin activation property, when the activity of somatostatin degrading enzyme in the nerve cells is suppressed due  
35 to a pretreatment with a certain compound, as compared to when the pretreatment with the compound is void.

With regard to the potentiation property of the somatostatin signal transmission, the potentiation property of the somatostatin

signal transmission is said to be expressed and the compound is said to have a brain somatostatin activation property, when the amount of intracellular phosphorylated protein in the nerve cells changes due to a pretreatment with a certain compound, as compared to when the pretreatment with the compound is void.

With regard to the property of suppressing the negative feedback mechanism of brain somatostatin release, the property of suppressing the negative feedback mechanism of brain somatostatin release is said to be expressed and the compound is said to have a brain somatostatin release promoting property, when the suppressive action on the membrane potential dependent calcium current by somatostatin in the hippocampus pyramidal cells is released due to a pretreatment with a certain compound.

Agent for expression of long-term potentiation of synaptic transmission

The compound having a brain somatostatin activation property expresses the long-term potentiation of synaptic transmission.

Therefore, this compound is used as an agent for expression of long-term potentiation of synaptic transmission for mammals such as human, dog, cow, horse, rat, guinea pig and the like.

In the present invention, an agent for expression of long-term potentiation of synaptic transmission means a compound capable of inducing potentiation of general neurotransmission efficiency after high frequency and short time stimulation and sustaining the increased transmission efficiency for a long time.

The site where the long-term potentiation of synaptic transmission is expressed is subject to no limitation as long as it is a nerve system present in the hippocampus. Examples thereof include nerve systems present in brain, such as cerebral cortex, corpus amygdaloideum and the like, with preference given to the mossy fiber - CA3 pathway, perforant path-dentate gyrus pathway and Schaffer collaterals-CA1 pathway.

The agent for expression of long-term potentiation of synaptic transmission of the present invention is effective for the prophylaxis and/or treatment of cerebral diseases such as dementia (e.g., senile dementia, Alzheimer's dementia, dementia associated with various diseases such as cerebral vascular dementia, cerebral post-traumatic dementia, dementia due to brain tumor, dementia due to chronic subdural hematoma, dementia due to normal pressure hydrocephalus, post-meningitis dementia, Parkinson's disease type dementia, and the

like), amnesia, manic-depressive psychosis, schizophrenia, Parkinson's disease, psychosomatic disease, and the like, particularly for the prophylaxis and/or treatment of dementia and amnesia.

The long-term potentiation of synaptic transmission is evaluated according to the following criteria.

In a long-term potentiation phenomenon observed in the hippocampus of an animal, namely, guinea pig, which is observed according to the method conventionally known (Matsuoka et al., *Brain Research*, vol. 553, p. 188, 1991), when a pretreatment of hippocampus with a certain compound (bringing hippocampus into contact with the compound) leads to the augmentation of LTP, after tetanic stimulation, of potential generated from the CA3 field pyramidal cell caused by stimulation of mossy fiber to not less than 120%, preferably not less than 140%, more preferably not less than 160%, still more preferably not less than 180%, most preferably not less than 200%, when that without the pretreatment with the compound is 100%, the long-term potentiation of synaptic transmission is said to be expressed, and this compound can be said to be an agent for expression of long-term potentiation of synaptic transmission.

Alternatively, in a long-term potentiating phenomenon observed in the hippocampus of an animal, namely, guinea pig, which is observed according to the method conventionally known (Matsuoka et al., *Brain Research*, vol. 553, p. 188, 1991), when a long-term potentiation phenomenon is expressed in a sustained manner for not less than 10 minutes, preferably not less than 20 minutes, more preferably not less than 30 minutes, most preferably not less than 60 minutes due to a pretreatment with a certain compound, a long-term potentiation of synaptic transmission is said to be expressed and this compound can be said to be an agent for expression of long-term potentiation of synaptic transmission.

Note that variations in numerical values to the degree that those of ordinary skill in the art consider substantially the same statistically as the above-mentioned numerical values should be construed as corresponding to the above-mentioned numerical values.

As used herein, the pretreatment with a certain compound can be conducted by, for example, immersing the compound generally for 25 minutes in an outer solution containing hippocampus therein, and removing the compound by a method such as washing.



The test method for the evaluation of the above-mentioned long-term potentiation of synaptic transmission may be a method conventionally known or a method analogous thereto, with preference given to the following method.

5 Round slices are prepared from the hippocampus removed from an animal, for example, guinea pig. The slices are 500  $\mu\text{m}$  in thickness. Each slice is placed in a container such as a perfusion chamber and the population spikes are extracellularly recorded in the following manner while perfusing with an artificial cerebrospinal fluid at 33  
10 - 34°C at a flow rate of 1.8 - 2.0 ml/min.

At this time, the test compound is added to the perfusate so that the concentration of the test compound in each container would be a serially diluted concentration, and the test compound is applied to the hippocampal slices from 21 minutes before the tetanic stimulation  
15 to 4 minutes thereafter.

The artificial cerebrospinal fluid to be used here is exemplified by those used in the screening method in the present invention to be mentioned later.

Mossy fiber is stimulated with a stimulation electrode at a voltage  
20 of not more than 10 V, frequency of 0.2 Hz, and the population spikes in the CA3 field pyramidal cell layer are recorded every 5 minutes. When the population spikes to be recorded are stabilized, tetanic stimulation is applied for the induction of LTP. The tetanic stimulation includes stimulation at the same voltage and a frequency of 33 Hz for  
25 5 seconds. The height of from the negative peak to the next positive peak of the obtained population spikes after stimulation is measured and taken as an amplitude of population spikes. The degree of LTP can be expressed by a potential variation (%) showing an increase in the amplitude of population spikes after tetanic stimulation relative to  
30 the average of 4 measurements of the amplitudes of population spikes before tetanic stimulation. As the index of the property of a drug, the area in the graph of time lapse versus potential variation, between the graph from 12 minutes to 62 minutes after tetanic stimulation and the line where the potential variation is 0%, may be calculated and  
35 taken as the magnitude of LTP.

Each value of the potential variation and the magnitude of LTP which is calculated based on the potential variation as mentioned above is subjected to one-way analysis of variance and Dunnett's multiple

comparison test, and compared based on the above-mentioned evaluation criteria, whereby the long-term potentiation of synaptic transmission can be evaluated.

**screening method**

5           The screening method of the present invention comprises screening of the test compound to be screened (hereinafter to be abbreviated as a test compound) using the somatostatin releasing property as an index.

10           The screening method of the agent for expression of long-term potentiation of synaptic transmission of the present invention, particularly, an anti-dementia agent and an anti-amnesia agent, is a selection method of the test compound characteristically comprising bringing human or animal nerve cells into contact with a test compound, measuring an amount of somatostatin released from the nerve cells and  
15           a release time thereof, measuring an amount of somatostatin released from the nerve cells and a release time thereof in the absence of a contact with the test compound, and comparing the amounts and the times to measure the amount of somatostatin released from the nerve cells and the release time thereof caused by the contact with the test compound.

20           The above-mentioned nerve cells include, for example, hippocampus, preferably hippocampal slices, primary culture nerve cell, nerve cell strain, oocyte and the like.

          The contact with the test compound can be conducted by, for example, immersing the test compound for 20 - 120 minutes in an outer solution  
25           in which the nerve cells are immersed and removing the test compound by washing and the like.

          Preferred screening method is as follows.

          Hippocampal slices are prepared from an animal (e.g., rat, guinea pig, mouse and the like) by a method conventionally known or a method  
30           analogous thereto. The slices are generally 100 - 600  $\mu\text{m}$ , preferably 300 - 400  $\mu\text{m}$ , in thickness. While the direction of slicing is not particularly limited, they are preferably transverse slices or longitudinal slices. For example, the slices are placed in a container such as a perfusion chamber, by generally 30 slices, more preferably  
35           20 slices, and perfused with an artificial cerebrospinal fluid while incubating at generally 20 - 37°C, preferably 36 - 37°C. The perfusate is exchanged every 5 - 20 minutes, preferably 10 - 15 minutes. Fractions are obtained from each cycle, which step is repeated 10 to 20 times,

preferably 15 times. While repeatedly obtaining the fractions, the test compound is added to the perfusate to serially diluted concentrations that are within the range of preferably  $10^{-10}$  -  $10^{-5}$  M, more preferably  $10^{-9}$  -  $10^{-6}$  M, whereby fractions containing the test compound at respective concentrations are obtained. The test compound is immersed in perfusate for generally 20 - 120 minutes, preferably 80 minutes.

Generally 0 - 60 minutes, preferably 10 - 30 minutes, after the addition of the test compound, stimulation is preferably added. The stimulation is applied for generally 5 - 20 minutes, preferably 10 - 15 minutes. It is important that the stimulation be applied after the addition of the test compound, but the stimulation may be applied while the test compound is present in the perfusate or after the test compound is removed by washing with a perfusate.

The somatostatin in the perfusate of each fraction obtained as above is quantified according to a treatment method conventionally known (for example, perfusate is lyophilized and subjected to a radioimmunoassay). After the completion of the step for obtaining the fractions, the somatostatin remaining in hippocampal slices is extracted by a conventional method, which amount is similarly quantified.

The composition of the artificial cerebrospinal fluid to be used as the perfusate in the above-mentioned screening method can be modified as appropriate depending on the test conditions and test compound and the like to be used. Preferable composition includes the following. Artificial cerebrospinal fluid composition: NaCl, 124 mM; KCl, 5 mM;  $\text{KH}_2\text{PO}_4$ , 1.24 mM;  $\text{MgSO}_4$ , 1.3 mM;  $\text{CaCl}_2$ , 2.4 mM;  $\text{NaHCO}_3$ , 26 mM; D-glucose, 10 mM

This perfusate is used with saturation with a mixed gas of oxygen and carbon dioxide, such as a mixed gas of oxygen (95%) and carbon dioxide (5%), typically used for pharmacological experiments.

The above-mentioned screening method may be modified as necessary.

The amount of somatostatin quantified is compared with the amount when the contact with the test compound is void, based on the following criteria, whereby the somatostatin release promoting property of the test compound can be evaluated.

The screening method of the present invention is preferably

conducted under stimulation as mentioned above. Such stimulation is a model of a specific stimulation related to learning or tetanic stimulation. The stimulation is not particularly limited as long as the nerve cells present in the hippocampal slice are excited. Specific  
5 examples thereof include stimulation by potassium ion, electric stimulation, depolarization stimulation, stimulation with a drug and the like. When a mere addition of the test compound does not lead to the somatostatin release property and the test compound shows  
10 somatostatin release property only upon stimulation, it can be a confirmation that the nerve cells are free from influence of this test compound as long as no stimulation is involved, thus ensuring the safety of this compound.

The somatostatin release property is evaluated based on the following criteria.

15 In the screening method of the present invention, when the somatostatin release amount due to the contact with the test compound increases by not less than 10%, preferably not less than 20%, more preferably not less than 30%, still more preferably not less than 40%, most preferably not less than 50%, as compared to the release amount  
20 when the contact with the test compound is void (which is taken as 100%), the test compound is said to have a somatostatin release promoting property. It is preferable that the somatostatin release amount upon stimulation mentioned above be evaluated according to the above-mentioned criteria. It is appreciated that variations in  
25 numerical values to the degree that those of ordinary skill in the art consider substantially the same statistically as the above-mentioned numerical values should be construed as corresponding to the above-mentioned numerical values.

The test compound to be subject to the screening in the present  
30 invention is free of any particular limitation and may be selected from natural product, chemically synthesized compound, nucleic acid, peptide, antibody and the like obtained by genetic engineering and their libraries. The test compound is preferably a pure substance, but may be a mixture or racemic compound. The test compound may be  
35 also modified to label with radioisotope or may contain modification made during construction of library. The obtained test compound can be optimized by chemical synthetic method and the like.

By selecting the test compounds using the screening method of

the present invention, an agent for expression of long-term potentiation of synaptic transmission, particularly an anti-dementia agent, an anti-amnesia agent and the like can be screened.

5 The test compound selected by the screening method of the present invention and a compound obtained by optimizing this compound are all encompassed in the scope of the present invention.

The compound of the present invention having a brain somatostatin activation property can be used in the dosage form of a solid, semi-solid or liquid preparation in conjunction with organic or inorganic carrier  
10 or excipient, which is suitable for rectal administration, pulmonary (pernasal or buckle inhalation), nasal drop, eyedrop, external (local), oral or parenteral (subcutaneous, intravenous or intramuscular) administration and the like, direct administration to diseased region, such as brain, spinal fluid, cerebroventricle and the like, or  
15 inhalation.

A compound having a brain somatostatin activation property can be admixed with pharmaceutically acceptable substantially non-toxic carrier or excipient conventionally used for dosage forms suitable for use, such as tablets, pellets, troches, capsules, suppositories,  
20 cream, ointment, aerosol, inhalable powder medicine, liquid, emulsion, suspension, and the like. Where necessary, auxiliary, stabilizer, tackifier, coloring agent and flavor can be used.

The agent for the expression of long-term potentiation of synaptic transmission, particularly an anti-dementia agent and an anti-amnesia  
25 agent, of the present invention can be produced by a method conventionally used in the pertinent field. Where necessary, a method routinely used in this technical field can be used for the production of these drugs for an improved bioavailability.

The agent for the expression of long-term potentiation of synaptic transmission, particularly an anti-dementia agent and an anti-amnesia  
30 agent, of the present invention is preferably administered intravenously (inclusive of addition into infusion), intramuscularly or orally when applying to humans or animals.

The agent for the expression of long-term potentiation of synaptic  
35 transmission, particularly an anti-dementia agent and an anti-amnesia agent, of the present invention is contained in a preparation in an amount sufficient to provide a desired prophylactic and/or treatment effect on the progression and conditions of diseases.

The amount and administration route of the compound having a brain somatostatin activation property are subject to variation depending on the kind of compound, age and conditions of the patients to be the subject of the prophylaxis and/or treatment. When compound 1 is used, for example, the daily dose is 0.1 - 10 mg/kg body weight by oral administration, which is given once to several times a day for the treatment and/or prophylaxis of the aforementioned diseases.

The present invention is explained in more detail in the following by way of Examples that do not limit the present invention.

10

#### Examples

**Experimental Example 1: Effect of compound 1 on somatostatin release from rat hippocampal slice**

##### (1) method

Rat hippocampal slices (thickness 350  $\mu$ m, round slice) were prepared by a standard method. Twenty rat hippocampal slices were placed in a perfusion chamber, incubated at 37°C and perfused by a batch method while exchanging the incubation buffer every 10 minutes. The incubation buffer used had the composition as noted below. A mixed gas of oxygen (95%) and carbon dioxide (5%) was used to saturate the buffer.

Composition of incubation buffer: NaCl, 124 mM; KCl, 5 mM;  $\text{KH}_2\text{PO}_4$ , 1.24 mM;  $\text{MgSO}_4$ , 1.3 mM;  $\text{CaCl}_2$ , 2.4 mM;  $\text{NaHCO}_3$ , 26 mM; D-glucose, 10 mM

Perfusion for 150 minutes gave fractions 1-15. To fraction 9 was applied a high  $\text{K}^+$  (50 mM) stimulation. Compound 1 was added to fractions 7 - 15 to the concentration of  $10^{-9}$  M,  $10^{-8}$  M,  $10^{-7}$  M,  $10^{-6}$  M, respectively. Nothing was added to control group. The respective fractions thus obtained were concentrated by lyophilization and somatostatin in the perfusate was quantified by radioimmunoassay (RIA). After the completion of the experiment, somatostatin remaining in the slices was extracted by a conventional method and quantified by radioimmunoassay. The somatostatin amount released by high  $\text{K}^+$  (50 mM) stimulation was calculated and the amount of somatostatin released due to the property of compound 1 was measured.

Somatostatin release (%) by compound 1 at each concentration was calculated as in the following. The somatostatin amount of each fraction was expressed by the percentage (%) relative to the somatostatin residual amount at the time the fraction was obtained. The value of fraction 8 immediately before high  $\text{K}^+$  (50 mM) stimulation was taken as the base and the values exceeding the base value were added with

regard to fraction 9 and the subsequent peak fractions exceeding the base value to give somatostatin release (%). The number of the test samples measured was 10 or 11. Each value (%) was expressed by mean  $\pm$  S.E.M. The property of compound 1 was subjected to Dunnett's multiple comparison test relative to control group.

## (2) Result

The results are shown in Fig. 1. The compound 1 promoted somatostatin release when stimulated at high  $K^+$  (50 mM), and the property was significant at  $10^{-7}$  M and  $10^{-6}$  M. The concentration dependency was similar to the long-term potentiation in Experimental Example 2 to be mentioned later. By the use of the screening method of the present invention, compound 1 was shown to have a somatostatin release promoting property on hippocampus.

**Experimental Example 2: Effect of compound 1 on LTP in mossy fiber - CA3 field pyramidal cell of hippocampal slice**

### (1) method

Slices (thickness 500  $\mu$ m, round slice) were prepared from hippocampus removed from male Hartley guinea pigs (body weight 220 - 350 g) and population spikes were extracellularly recorded. The hippocampal slices were perfused with an artificial cerebrospinal fluid (33-34°C, composition: NaCl, 124 mM; KCl, 5 mM;  $KH_2PO_4$ , 1.24 mM;  $MgSO_4$ , 1.3 mM;  $CaCl_2$ , 2.4 mM;  $NaHCO_3$ , 26 mM; D-glucose, 10 mM) saturated with a mixed gas of oxygen (95%) and carbon dioxide (5%) at flow rate of 1.8 - 2.0 ml/min. Mossy fiber was stimulated with a stimulating electrode at a voltage of not more than 10 V, frequency of 0.2 Hz and the population spikes in the CA3 field pyramidal cell layer was recorded every 5 minutes. When the population spikes to be recorded were stabilized, tetanic stimulation for induction of LTP was applied. The tetanic stimulation includes stimulation at the same voltage of not more than 10 V, as when the stimulation was applied at a frequency of 0.2 Hz, but upon increase of the frequency to 33 Hz for 5 seconds. The height of from negative peak to the next positive peak of the obtained population spikes after stimulation was measured and taken as an amplitude (population spike amplitude; PSA). The degree of LTP was expressed by a potential variation (%) by the increase in the amplitude of population spikes after tetanic stimulation relative to the average of 4 measurements of the amplitudes of population spikes obtained before tetanic stimulation. Each value was expressed by mean  $\pm$  S.E.M (the

number of slices tested was 3 to 8). As the index of the property of a drug, the area (%·min) between the graph from 12 minutes to 62 minutes after tetanic stimulation and the line where the potential variation is 0%, in the time lapse graph of potential variation, was calculated and taken as the magnitude of LTP. Compound 1 was dissolved in and diluted with distilled water and added to perfusate to the concentration of  $10^{-9}$  M,  $10^{-8}$  M,  $10^{-7}$  M and  $10^{-6}$  M, respectively, and applied to a hippocampal slice for 25 minutes from 2 minutes before the tetanic stimulation to 4 minutes thereafter. Nothing was added to perfusate for control group.

The property of compound 1 relative to the magnitude of LTP was tested by one-way analysis of variance and Dunnett's multiple comparison test.

## (2) Results

From the potential variation with the lapse of time, compound 1 was found to have shown no significant influence of the response of base before tetanic stimulation. As is can be seen from Fig. 2 showing the magnitude of LTP, compound 1 increased potential variation after tetanic stimulation at  $10^{-7}$  M and  $10^{-6}$  M, wherein its action was maximal at  $10^{-7}$  M. From the foregoing, it was shown that compound 1 had a promoting property specific to the mechanism necessary for the occurrence of phenomenon of long-term potentiation of synaptic transmission after tetanic stimulation.

The hippocampal slice obtained from the guinea pig treated with cysteamine (200 mg/kg) did not show LTP enhancing property by compound 1 at  $10^{-7}$  M. This reveals that hippocampus endogeneous somatostatin activation property is involved in the LTP enhancing property by compound 1.

**Experimental Example 3: Effect of compound 1 on voltage-dependent  $\text{Ca}^{2+}$  current of rat hippocampus pyramidal cell**

### (1) method

Hippocampal slices were prepared from the brains of 5- to 14-day-old male Wistar rats and the nerve cells were quickly isolated by trypsin enzyme treatment method. According to the whole cell patch-clamp method, the membrane potential of hippocampus pyramidal cell was fixed at -80 mV and the membrane potential-dependent potassium current ( $I_{\text{K}}$ ) was measured in  $\text{Cs}^+$  electrode inner solution and  $\text{Ba}^{2+}$  bath outer solution. The resistance of the electrode was 2 - 4 M $\Omega$ .



Compound 1 was added to bath outer solution concentrations of 0.01  $\mu\text{M}$ , 0.1  $\mu\text{M}$  and 1.0  $\mu\text{M}$ , respectively, and perfused with the bath outer solution. Nothing was added to the bath outer solution for the control group. The maximal value of the current (peak amplitude) was measured, and calculated as the variation (%) relative to the value before addition of compound 1. Each value (%) was expressed by  $\text{mean} \pm \text{S.E.M.}$ . The number of measurements was 7. The property of compound 1 was evaluated by Dunnett's multiple comparison test relative to the control group.

(2) Result

The results are shown in Fig. 3. Compound 1 significantly promoted membrane potential-dependent calcium current at concentrations of 0.01  $\mu\text{M}$ , 0.1  $\mu\text{M}$  and 1.0  $\mu\text{M}$  as compared to the control group, and showed bell-shape dose dependency. Therefore, compound 1 was shown to have a promoting action on the voltage-dependent calcium channel of hippocampus pyramidal cells.

**Experimental Example 4: Effect of somatostatin and compound 1 on voltage-dependent  $\text{Ca}^{2+}$  current of rat hippocampus pyramidal cell**

(1) method

In the same manner as in Experimental Example 3 except that compound 1 and somatostatin were added in such a manner that the concentration of somatostatin in the bath outer solution was  $10^{-7}$  M when somatostatin alone was added, and the concentration of compound 1 in the bath outer solution was  $10^{-7}$  M and the concentration of somatostatin in the bath outer solution was  $10^{-7}$  M when both compound 1 and somatostatin were added, the experiment was conducted. Nothing was added to the control group. The maximal value of each current was measured, and calculated as the variation (%) to that prior to addition. Each value (%) was expressed by  $\text{mean} \pm \text{S.E.M.}$ . The number of measurements of the group added with somatostatin was 11 and that of the group added with both compound 1 and somatostatin was 7. The both groups were subjected to Dunnett's multiple comparison test.

(2) Result

The results are shown in Fig. 4. While somatostatin evidently suppressed membrane potential-dependent calcium current, suppressive property of somatostatin was completely released in the presence of compound 1. Hence, compound 1 was shown to release the suppressive property of somatostatin on the voltage-dependent calcium channel of hippocampus pyramidal cell and has a promoting property. From the

results of Experimental Example 3 and this Experimental Example, compound 1 was shown to suppress the negative feedback mechanism of brain somatostatin release.

- 5           The compounds [II-1] including compounds [II-2] used in the present invention and preparation methods thereof are explained in detail by way of the following Reference preparations and Reference examples. It is needless to say that those Reference preparations and Reference examples do not limit the present invention.

10   Reference preparation 1

- To a solution of 1-benzyl-4-aminopiperidine (50 g) in water (360 ml) was added a solution of di-tert-butyl dicarbonate (61 g) in acetone (360 ml) dropwise under cooling on an ice-water bath. After stirring for 2.5 hours, a precipitate was collected on a filter, washed with  
15   water, and dried. The crude product was poured into a mixture of diisopropyl ether (200 ml) and n-hexane (200 ml) and the mixture was stirred. After filtration, O-tert-butyl N-(1-benzylpiperidin-4-yl)-carbamate (66.9 g) was obtained.

- 20           NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.2-1.5 (2H, m), 1.37 (9H, s), 1.66 (2H, br d, J=9.9Hz), 1.91 (2H, br t J=10.7Hz), 2.73 (2H, distorted d, J=11.8Hz), 3.2 (1H, m), 3.41 (2H, s), 6.75 (1H, d, J=7.8Hz), 7.1-7.4 (5H, m)

          MASS (APCI)(m/z): 291

Reference preparation 2

- 25           To a mixture of O-tert-butyl N-(1-benzylpiperidin-4-yl)-carbamate (45 g) and 10% palladium on carbon (50% wet, 9 g) in methanol (1 l) was bubbled hydrogen gas under stirring at ambient temperature. The catalyst was removed by glass filter and the solvent was removed under reduced pressure. After rinse with diisopropyl ether,  
30   O-tert-butyl N-(piperidin-4-yl)carbamate (28.35 g) was obtained. The washed solvent was removed under reduced pressure, and the residue was rinsed with diisopropyl ether. The second fraction of O-tert-butyl N-(piperidin-4-yl)carbamate (344 mg) was obtained.

- 35           NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.18 (2H, ddd, J=3.8, 11.8, 11.8Hz), 1.37 (9H, s), 1.62 (2H, distorted d, J=10.8Hz), 1.85 (1H, m), 2.38 (2H, dt, J=2.2, 12.0Hz), 2.86 (2H, distorted d, J=12.3Hz), 3.2 (1H, m), 6.72 (1H, br d)

          MASS (APCI)(m/z): 201

Reference preparation 3

To a suspension of O-tert-butyl N-(piperidin-4-yl)carbamate (4.0 g) in dichloromethane (40 ml) were added pyridine (1.94 ml), dichloromethane (40 ml), acetic anhydride (20.8 ml) and then  
5 N,N-dimethylaminopyridine (0.1 g) at ambient temperature. After stirring for 3 hours, the mixture was washed with 0.1N hydrochloric acid, water, and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. After rinse with diisopropyl ether, O-tert-butyl N-(1-acetylpiperidin-4-yl)carbamate  
10 (4.01 g) was obtained.

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.23 (2H, m), 1.38 (9H, s), 1.70 (2H, distorted t, J=11.4Hz), 1.97 (3H, s), 2.64 (1H, br t, J=11.1Hz), 3.04 (1H, dt, J=2.8, 11.5Hz), 3.42 (1H, m), 3.72 (1H, br d, J=15.0Hz), 4.19 (1H, br d, J=13.1Hz), 6.86 (1H, d, J=7.5Hz)

15 MASS (APCI)(m/z): 243

Reference preparation 4

To a solution of O-tert-butyl N-(1-acetylpiperidin-4-yl)-carbamate (2.42 g) in dichloromethane (24 ml) was added 4N hydrogen chloride in dioxane (24 ml). The solvents were removed under reduced  
20 pressure. After rinse with diisopropyl ether, 1-acetyl-4-aminopiperidine hydrochloride (2.02 g) was obtained.

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.41 (2H, m), 1.93 (2H, distorted t), 2.00 (3H, s), 2.60 (1H, br t, J=10.4Hz), 3.06 (1H, br t, J=11.3Hz), 3.12 (1H, m), 3.84 (1H, br d, J=14.0Hz), 4.34 (1H, br d, J=13.0Hz),  
25 8.32 (3H, br s)

MASS (APCI)(m/z): 143

Reference preparation 5

To a solution of phenyl chloroformate (5.64 g) in dichloromethane (70 ml) was added a solution of 4-aminopyridine (2.84 g) and triethylamine (5.02 ml) in dichloromethane (100 ml) dropwise under cooling on an ice-water bath. After stirring for 1 hour, the solvents were removed under reduced pressure. A residue was diluted with dichloromethane (200 ml) and water (200 ml). An organic phase was separated and washed with water and brine. After drying with magnesium sulfate, the solvents  
30 were removed under reduced pressure. The reaction mixture was diluted with diisopropyl ether and the precipitates were filtered. After rinse with diethyl ether, O-phenyl N-(4-pyridyl)carbamate (5.07 g) was obtained.

NMR (CDCl<sub>3</sub>,  $\delta$ ): 7.17 (2H, m), 7.27 (1H, m), 7.3-7.5 (4H, m), 8.50 (2H, dd, J=1.4, 5.0Hz), 8.06 (1H, s)

MASS (APCI)(m/z): 215

Reference preparation 6

- 5 A solution of sulfuryl chloride (3.55 ml) in chloroform (45 ml) was added a solution of 1-acetylpiperazine (5.66 mg) and triethylamine (6.16 ml) in chloroform (15 ml) dropwise under cooling on an ice-water bath. After stirring for 6 hours, a precipitate was collected by filtration. After drying over sodium hydroxide, 1-acetylpiperazine-4-sulfonyl chloride (2.43 g) was obtained.

NMR (CDCl<sub>3</sub>,  $\delta$ ): 2.15 (3H, s), 3.35 (4H, m), 3.69 (2H, t, J=5.1Hz), 3.83 (2H, br s)

MASS (APCI)(m/z): 227

Reference preparation 7

- 15 To a solution of 1-benzyl-4-aminopiperidine (1.13 g) in dichloromethane (10 ml) were added a solution of 4-fluorobenzoyl chloride (0.99 g) in dichloromethane (1 ml) and diisopropylethylamine (1.09 ml) under cooling on an ice-water bath. The mixture was warmed to ambient temperature slowly under stirring. The mixture was diluted with dichloromethane and washed with water, saturated aqueous sodium hydrogen carbonate, water, and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 100 ml, dichloromethane:methanol = 15:1). After rinse with diisopropyl ether - n-hexane (1:1), N-(1-benzylpiperidin-4-yl)-4-fluorobenzamide (1.31 g) was obtained.

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.4-1.7 (2H, m), 1.7-1.9 (2H, m), 2.01 (2H, br t, J=10.7Hz), 2.81 (2H, br d, J=11.6Hz), 3.46 (2H, s), 3.73 (1H, m), 7.2-7.4 (7H, m), 7.90 (2H, dd, J=5.6, 8.9Hz), 8.26 (1H, br d, J=7.7Hz)

MASS (APCI)(m/z): 313

Reference preparation 8

- The following compound was obtained by using 4-amino-1-benzylpiperidine as a starting compound according to a similar manner to that of Reference example 2.

N-(1-Benzylpiperidin-4-yl)-N'-(4-fluorophenyl)urea

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.25-1.5 (2H, m), 1.7-1.9 (2H, m), 2.0-2.2 (2H, m), 2.65-2.8 (2H, m), 3.4-3.6 (3H, m), 6.07 (1H, d, J=7.6Hz),

7.05 (2H, t, J=9Hz), 7.2-7.45 (2H, m), 8.35 (1H, s)

MASS (APCI)(m/z): 328

Reference preparation 9

To a solution of N-(1-benzylpiperidin-4-yl)-N'-(4-fluoro-phenyl)urea (3.0 g) in a mixture of methanol (15 ml) and tetrahydrofuran (15 ml) was added palladium on carbon (10% w/w, 50% wet, 0.6 g), and the mixture was hydrogenated under atmospheric pressure of hydrogen for 8 hours. The catalyst was filtered off, and the solvents were evaporated under reduced pressure to give a residue, which was triturated with diisopropyl ether to give N-(piperidin-4-yl)-N'-(4-fluorophenyl)urea (1.97 g).

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.1-1.4 (2H, m), 1.65-1.85 (2H, m), 2.3-2.65 (2H, m), 2.8-3.0 (2H, m), 3.3-3.7 (1H, m), 6.08 (1H, d, J=8Hz), 7.04 (2H, t, J=9Hz), 7.25-7.5 (2H, m), 8.33 (1H, s)

MASS (APCI)(m/z): 238

Reference preparation 10

A mixture of N-(1-benzylpiperidin-4-yl)-4-fluorobenzamide (937 mg) and 10% palladium on carbon (50% wet, 0.2 g) in methanol (20 ml) was stirred under hydrogen atmosphere for 7.5 hours at ambient temperature. The catalyst was removed by glass filter and the solvent was removed under reduced pressure. After rinse with diisopropyl ether, N-(piperidin-4-yl)-4-fluorobenzamide (653 mg) was obtained.

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.40 (2H, ddd, J=4.0, 11.9, 23.8Hz), 1.72 (2H, br d, J=9.5Hz), 2.3-2.7 (2H, m), 2.8-3.2 (2H, m), 3.80 (1H, m), 7.27 (2H, t, J=8.9Hz), 7.92 (2H, dd, J=5.6, 8.9Hz), 8.26 (1H, d, J=7.7Hz)

MASS (APCI)(m/z): 223

Reference example 1

To a solution of O-phenyl N-(4-pyridyl)carbamate (446 mg) in 1,2-dichloroethane (5 ml) was added a suspension of 1-acetylpiperazine (1.12 g) in 1,2-dichloroethane (20 ml) at ambient temperature. The mixture was heated at 60°C with stirring for 9 hours. The mixture was cooled to ambient temperature, and diluted with dichloromethane and water. The aqueous phase was separated and adjusted to pH 11.5 with sodium hydroxide solution. Excess sodium chloride was added to the aqueous solution. The mixture was extracted with a mixture of dichloromethane and methanol (about 10:1) and the organic phase was washed with brine. After drying with magnesium sulfate, the solvents

were removed under reduced pressure. A residue was purified by column chromatography (silica gel 100 ml, dichloromethane:methanol:aqueous ammonia = 10:1:0.1). After rinse with diisopropyl ether, 1-acetyl-4-(4-pyridylaminocarbonyl)piperazine (398 mg) was obtained.

5 NMR (DMSO- $d_6$ ,  $\delta$ ): 2.03 (3H, s), 3.3-3.6 (8H, m), 7.47 (2H, dd, J=1.5, 4.8Hz), 8.31 (2H, dd, J=1.5, 4.8Hz), 9.01 (1H, s)

MASS (APCI)(m/z): 271

#### Reference example 2

To a stirred solution of 1-acetylpiperazine (0.648 g) in tetrahydrofuran (10 ml) was added 4-fluorophenyl isocyanate (0.574 g) at ambient temperature. After stirring at ambient temperature for 1 hour, the solvent was removed by evaporation under reduced pressure, and the residue was triturated with diisopropyl ether to give 1-acetyl-4-(4-fluorophenylcarbonyl)piperazine (1.25 g).

15 NMR (DMSO- $d_6$ ,  $\delta$ ): 2.03 (3H, s), 3.3-3.6 (8H, m), 7.07 (2H, t, J=9Hz), 7.46 (2H, dd, J=5, 9Hz), 8.61 (1H, s)

MASS (APCI)(m/z): 266

#### Reference example 3

The following compound was obtained by using 1-tert-butoxycarbonylpiperazine as a starting compound according to a similar manner to that of Reference example 2.

1-tert-Butoxycarbonyl-4-(4-fluorophenylcarbonyl)piperazine

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.42 (9H, s), 3.25-3.5 (8H, m), 7.07 (2H, t, J=9Hz), 7.45 (2H, dd, J=5, 9Hz), 8.60 (1H, s)

25 MASS (LD)(m/z): 346.2

#### Reference example 4

To a solution of pyridine-4-carboxylic acid (1.0 g) and triethylamine (1.2 ml) in toluene (20 ml) was added diphenylphosphoryl azide (1.75 ml) at ambient temperature. The resulting mixture was heated to reflux for 30 minutes and cooled to 0°C. To the mixture was added 1-tert-butoxycarbonylpiperazine (1.51 g), and the mixture was allowed to heat to 90°C for 1 hour. After cooling to ambient temperature, the reaction mixture was taken up into ethyl acetate, washed in turn with water and brine, dried over magnesium sulfate, and evaporated under reduced pressure. The residue was chromatographed on silica gel (150 ml) eluting with 0-7% methanol in dichloromethane. Trituration with a mixture of diisopropyl ether and ethanol gave 1-tert-butoxycarbonyl-4-(pyridin-4-ylcarbonyl)piperazine (0.66 g).

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.42 (9H, s), 3.25-3.5 (8H, m), 7.46 (2H, d,  $J=1.5$ , 5Hz), 8.30 (2H, d,  $J=1.5$ , 5Hz), 9.00 (1H, s)

MASS (LD)(m/z): 307.2

Reference example 5

5 To a suspension of 1-acetyl-4-aminopiperidine hydrochloride (0.4 g) in dichloromethane (5 ml) were added in turn pyridine (0.54 ml) and 4-fluorophenyl chloroformate (0.29 ml) at 0°C. The mixture was allowed to warm to ambient temperature and stirred for 1 hour, which was taken up into a mixture of water and ethyl acetate. The separated  
10 organic layer was washed in turn with hydrochloric acid (1N), aqueous sodium hydrogen carbonate, and brine, and dried over magnesium sulfate. Evaporation under reduced pressure gave a residue, which was triturated with diisopropyl ether to give 1-acetyl-4-(4-fluorophenoxycarbonyl-amino)piperidine (347 mg).

15 NMR (DMSO- $d_6$ ,  $\delta$ ): 1.15-1.55 (2H, m), 1.7-1.95 (2H, m), 2.00 (3H, s), 2.65-2.85 (1H, m), 3.0-3.25 (1H, m), 3.5-3.7 (1H, m), 3.7-3.9 (1H, m), 4.15-4.3 (1H, m), 7.05-7.3 (4H, m), 7.86 (1H, d,  $J=8$ Hz)

MASS (APCI)(m/z): 281

Reference example 6

20 To a suspension of 1-acetyl-4-aminopiperidine hydrochloride (715 mg) in dichloromethane (7 ml) were added diisopropylethylamine (1.83 ml) and a solution of 4-fluorobenzoyl chloride (0.83 mg) in dichloromethane (2 ml) at ambient temperature. After stirring for 6.5 hours, the reaction mixture was diluted with dichloromethane and washed  
25 with water, saturated aqueous sodium hydrogen carbonate, and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 50 ml, dichloromethane:methanol = 50:1 to 10:1). After  
30 rinse with diisopropyl ether, N-(1-acetylpiperidin-4-yl)-4-fluorobenzamide (738 mg) was obtained.

35 NMR (DMSO- $d_6$ ,  $\delta$ ): 1.40 (2H, m), 1.81 (2H, distorted t,  $J=12.4$ Hz), 2.01 (3H, s), 2.68 (1H, br t,  $J=11.4$ Hz), 3.13 (1H, br t,  $J=11.6$ Hz), 3.83 (1H, br t,  $J=13.9$ Hz), 4.01 (1H, m), 4.33 (1H, br d,  $J=13.7$ Hz), 7.29 (2H, t,  $J=8.9$ Hz), 7.92 (2H, dd,  $J=5.5$ , 8.8Hz), 8.31 (1H, d,  $J=7.7$ Hz)

MASS (APCI)(m/z): 265

Reference example 7

To a suspension of 1-acetyl-4-aminopiperidine hydrochloride (536

mg) in dichloromethane (5 ml) were added isonicotinoyl chloride hydrochloride (534 mg) and diisopropylethylamine (1.05 ml) at ambient temperature. After stirring for 8 hours, the reaction mixture was poured into water and diluted with dichloromethane. The mixture was adjusted to pH 8.5 with 1N sodium hydroxide solution. Sodium chloride was added to the mixture and an organic phase was separated. The aqueous phase was extracted with dichloromethane and a combined organic phase was dried over magnesium sulfate. The solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 50 ml, dichloromethane:methanol = 10:1). After crystallization from diisopropyl ether:n-hexane, N-(1-acetylpiperidin-4-yl)-N-isonicotinamide (477 mg) was obtained.

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.4 (2H, m), 1.83 (2H, distorted t, J=11Hz), 2.01 (3H, s), 2.69 (1H, br t, J=11Hz), 3.14 (1H, br t, J=12Hz), 3.83 (1H, br d, J=14.1Hz), 4.03 (1H, m), 4.33 (1H, br d, J=13.1Hz), 7.75 (2H, dd, J=1.7, 4.4Hz), 8.62 (1H, d, J=7.5Hz), 8.72 (2H, dd, J=1.6, 4.4Hz)

MASS (APCI)(m/z): 248

#### Reference example 8

To a suspension of 1-acetyl-4-aminopiperidine hydrochloride (715 mg) in dichloromethane (7 ml) were added diisopropylethylamine (1.83 ml) and a solution of 4-fluorobenzenesulfonyl chloride (0.83 mg) in dichloromethane (2 ml) at ambient temperature. After stirring for 6.5 hours, the reaction mixture was diluted with dichloromethane and washed with water, saturated aqueous sodium hydrogen carbonate, and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 50 ml, dichloromethane:methanol = 50:1 to 20:1). After rinse with diisopropyl ether, N-(1-acetylpiperidin-4-yl)-4-fluorobenzenesulfonamide (859 mg) was obtained.

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.21 (2H, m), 1.54 (2H, m), 1.94 (3H, s), 2.66 (1H, br t, J=10.8Hz), 3.02 (1H, dt, J=2.9, 12.0Hz), 3.22 (1H, m), 3.64 (1H, br d, J=14.0Hz), 4.05 (1H, br d, J=13.2Hz), 7.44 (2H, t, J=8.9Hz), 7.8-8.0 (3H, m)

MASS (APCI)(m/z): 301

#### Reference example 9

To a solution of O-phenyl N-(4-pyridyl)carbamate (0.81 g) in chloroform (10 ml) were added 1-acetyl-4-aminopiperidine hydrochloride



(0.68 g) and triethylamine (1.06 ml) at ambient temperature. After stirring for 1 day, the mixture changed to a solution. The solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 100 ml, dichloromethane:methanol = 10:1 to 5:1, and silica gel 50 ml, dichloromethane:methanol:aqueous ammonia = 10:1:0.1). The solvents of desired fractions were removed under reduced pressure. A residue was dissolved with methanol (5 ml) and dichloromethane (5 ml), and 4N hydrogen chloride in dioxane (1.5 ml) was added to the solution. The solvents were removed under reduced pressure, and the residue was evaporated azeotropically with methanol. After crystallization from diisopropyl ether and n-hexane, N-(1-acetylpiperidin-4-yl)-N'-(4-pyridyl)urea (343 mg) was obtained.

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.1-1.6 (2H, m), 1.77 (2H, m), 2.01 (3H, s), 2.94 (1H, br t, J=10.4Hz), 3.22 (1H, br t, J=10.1Hz), 3.76 (2H, m), 4.05 (1H, d, J=13.6Hz), 7.60 (1H, d, J=7.8Hz), 7.83 (2H, d, J=6.8Hz), 8.52 (2H, d, J=7.1Hz), 11.21 (1H, s), 14.66 (1H, br s)

MASS (APCI)(m/z): 263

#### Reference example 10

To a suspension of 1-acetyl-4-aminopiperidine hydrochloride (536 mg) in dichloromethane (5 ml) were added 4-fluorophenyl isocyanate (375  $\mu$ l) and diisopropylethylamine (575  $\mu$ l) at ambient temperature. After stirring for 3 hours, the reaction mixture was diluted with dichloromethane. An organic phase was separated and an aqueous phase was extracted with dichloromethane. A combined organic phase was dried over magnesium sulfate and the solvents were removed under reduced pressure. After crystallization from diisopropyl ether and n-hexane, N-(1-acetylpiperidin-4-yl)-N'-(4-fluorophenyl)urea (448 mg) was obtained.

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.1-1.5 (2H, m), 1.80 (2H, distorted t, J=10Hz), 2.00 (3H, s), 2.77 (1H, br d, J=10.8Hz), 3.14 (1H, br d, J=11.1Hz), 3.5-3.9 (2H, m), 4.16 (1H, br d, J=13.2Hz), 6.15 (1H, d, J=7.6Hz), 7.05 (2H, t, J=8.9Hz), 7.40 (2H, dd, J=5.0, 9.2Hz), 8.37 (1H, s)

MASS (APCI)(m/z): 280

#### Reference example 11

To a solution of 4-(4-fluorobenzoylamino)piperidine (0.25 g) in dichloromethane (5 ml) were added in turn pyridine (0.14 ml) and methyl chloroformate (87  $\mu$ l) at 0°C. The mixture was allowed to warm

to ambient temperature and stirred for 1 hour. To the mixture was added N,N-dimethylaminopyridine (0.13 g) and allowed to stir for another 1 hour. The reaction mixture was taken up into a mixture of water and ethyl acetate. The separated organic layer was washed in turn with  
5 hydrochloric acid (1N), aqueous sodium hydrogen carbonate, and brine, and dried over magnesium sulfate. Evaporation under reduced pressure gave a residue, which was triturated with diisopropyl ether to give 4-(4-fluorobenzoylamino)-1-methoxycarbonylpiperidine (0.265 g).

10 NMR (DMSO- $d_6$ ,  $\delta$ ): 1.3-1.6 (2H, m), 1.75-1.9 (2H, m), 2.8-3.05 (2H, m), 3.60 (3H, s), 3.85-4.1 (2H, m), 7.29 (2H, t, J=9Hz), 7.90 (2H, dd, J=6, 9Hz), 8.30 (1H, d, J=8Hz)

MASS (APCI)(m/z): 281

Reference example 12

To a solution of 4-(4-fluorobenzoylamino)piperidine (0.25 g)  
15 in pyridine (5 ml) were added in turn 4-fluorobenzenesulfonyl chloride (0.219 g) and catalytic amount of N,N-dimethylaminopyridine at 0°C. The mixture was allowed to warm to ambient temperature and stirred for 1 hour, which was taken up into a mixture of water and dichloromethane. The separated organic layer was washed in turn with hydrochloric acid  
20 (1N), aqueous sodium hydrogen carbonate, and brine, and dried over magnesium sulfate. Evaporation under reduced pressure gave a residue, which was triturated with diisopropyl ether to give 4-(4-fluorobenzoylamino)-1-(4-fluorophenylsulfonyl)piperidine (0.38 g).

25 NMR (DMSO- $d_6$ ,  $\delta$ ): 1.45-1.7 (2H, m), 1.8-1.95 (2H, m), 2.35-2.55 (2H, m), 3.5-3.85 (3H, m), 7.28 (2H, t, J=9Hz), 7.50 (2H, t, J=9Hz), 7.75-7.95 (4H, m), 8.31 (1H, d, J=8Hz)

MASS (APCI)(m/z): 381

Reference example 13

30 To a solution of 4-(4-fluorobenzoylamino)piperidine (0.15 g) in dichloromethane (5 ml) were added in turn pyridine (82  $\mu$ l) and 4-trifluoromethoxybenzoyl chloride (106  $\mu$ l) at 0°C. The mixture was allowed to warm to ambient temperature and stirred for 4 hours, which was taken up into a mixture of water and dichloromethane. The separated  
35 organic layer was washed in turn with hydrochloric acid (1N), aqueous sodium hydrogen carbonate, and brine, and dried over magnesium sulfate. Evaporation of the solvent under reduced pressure gave 4-(4-fluorobenzoylamino)-1-(4-trifluoromethoxybenzoyl)piperidine

(205 mg).

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.3-1.7 (2H, m), 1.7-2.0 (2H, m), 2.7-3.4 (2H, m), 3.4-3.8 (1H, m), 3.9-4.2 (1H, m), 4.2-4.6 (1H, m), 7.30 (2H, t, J=9Hz), 7.35-7.6 (4H, m), 7.91 (2H, dd, J=6, 9Hz), 8.35 (1H, d, J=8Hz)

MASS (LD)(m/z): 433.2

Reference example 14

To a solution of 4-(4-fluorobenzoylamino)piperidine (0.15 g) in dichloromethane (5 ml) were added in turn pyridine (0.14 ml) and methanesulfonyl chloride (96  $\mu$ l) at 0°C. The mixture was allowed to warm to ambient temperature and stirred for 1 hour. To the mixture was added N,N-dimethylaminopyridine (0.13 g) and allowed to stir for another 1 hour. The reaction mixture was taken up into a mixture of water and dichloromethane. The separated organic layer was washed in turn with hydrochloric acid (1N), aqueous sodium hydrogen carbonate, and brine, and dried over magnesium sulfate. Evaporation under reduced pressure gave a residue, which was triturated with diisopropyl ether to give 4-(4-fluorobenzoylamino)-1-methylsulfonylpiperidine (0.30 g).

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.45-1.7 (2H, m), 1.8-2.05 (2H, m), 2.7-2.95 (2H, m), 2.88 (3H, s), 3.5-3.65 (2H, m), 3.8-4.05 (1H, m), 7.30 (2H, t, J=9Hz), 7.91 (2H, dd, J=6, 9Hz), 8.36 (1H, d, J=8Hz)

MASS (APCI)(m/z): 301

Reference example 15

To a solution of N-(piperidin-4-yl)-N'-(4-fluorophenyl)-urea (0.3 g) in tetrahydrofuran (4 ml) were added in turn pyridine (0.28 ml), methyl chloroformate (98  $\mu$ l) and catalytic amount of N,N-dimethylaminopyridine at 0°C. The mixture was allowed to warm to ambient temperature and stirred for 2 hours. The reaction mixture was taken up into a mixture of water and ethyl acetate. The separated organic layer was washed in turn with hydrochloric acid (1N), aqueous sodium hydrogen carbonate, and brine, and dried over magnesium sulfate. Evaporation under reduced pressure gave a residue, which was triturated with diisopropyl ether to give N-(1-methoxycarbonylpiperidin-4-yl)-N'-(4-fluorophenyl)urea (0.312 g).

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.1-1.4 (2H, m), 1.7-1.9 (2H, m), 2.8-3.1 (2H, m), 3.5-3.75 (1H, m), 3.59 (3H, s), 3.75-3.95 (2H, m), 6.15 (1H, d, J=7.6Hz), 7.05 (2H, t, J=9Hz), 7.37 (2H, dd, J=5, 9Hz), 8.37

(1H, s)

MASS (APCI)(m/z): 296

Reference example 16

To a solution of N-(piperidin-4-yl)-N'-(4-fluorophenyl)urea  
5 (0.3 g) in tetrahydrofuran (4 ml) were added in turn N,N-dimethylamino-  
pyridine (0.23 g) and 4-fluorobenzenesulfonyl chloride (0.25 g) at  
0°C. The mixture was allowed to warm to ambient temperature and stirred  
for 1 hour. The reaction mixture was taken up into a mixture of water  
and dichloromethane. The separated organic layer was washed in turn  
10 with hydrochloric acid (1N), aqueous sodium hydrogen carbonate, and  
brine, and dried over magnesium sulfate. Evaporation under reduced  
pressure gave a residue, which was triturated with diisopropyl ether  
to give N-(1-(4-fluorophenylsulfonyl)piperidin-4-yl)-N'-(4-fluoro-  
phenyl)urea (0.468 g).

15 NMR (DMSO-d<sub>6</sub>, δ): 1.3-1.6 (2H, m), 1.75-1.95 (2H, m), 2.45-2.7 (2H,  
m), 3.35-3.6 (3H, m), 6.14 (1H, d, J=7.5Hz), 7.03 (2H, t, J=9Hz),  
7.34 (2H, dd, J=5, 9Hz), 7.50 (2H, t, J=9Hz), 7.75-7.95 (2H,  
m), 8.31 (1H, s)

MASS (APCI)(m/z): 396

20 Reference example 17

To a suspension of N-(piperidin-4-yl)-4-fluorobenzamide (0.5  
g) in dichloromethane (5 ml) were added pyridine (218 μl),  
dichloromethane (5 ml) and benzoyl chloride (290 μl) at ambient  
temperature. After stirring for 3.5 hours, water (5 ml) was poured  
25 into the mixture. An organic layer was separated, and washed with water  
and brine. After drying with magnesium sulfate, the solvents were  
removed under reduced pressure. A residue was purified by column  
chromatography (silica gel, toluene:ethyl acetate = 1:1 to ethyl  
acetate). After rinse with diisopropyl ether,

30 N-(1-benzoylpiperidin-4-yl)-4-fluorobenzamide (515 mg) was obtained.

NMR (DMSO-d<sub>6</sub>, δ): 1.50 (2H, br s), 1.85 (2H, br s), 2.8-3.3 (2H,  
m), 3.61 (1H, m), 4.1 (1H, m), 4.35 (1H, m), 7.29 (2H, t, J=8.9Hz),  
7.3-7.5 (5H, m), 7.92 (2H, dd, J=5.6, 8.9Hz), 8.34 (1H, d, J=7.9Hz)

MASS (APCI)(m/z): 327

35 Reference example 18

To a suspension of N-(piperidin-4-yl)-4-fluorobenzamide (556  
mg) in dichloromethane (5 ml) were added pivaloyl chloride (0.37 ml),  
pyridine (0.24 ml) and N,N-dimethylaminopyridine (25 mg) at ambient

temperature. After stirring for 1 day, the mixture was diluted with dichloromethane, and washed with water and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. After trituration with diisopropyl ether, N-(1-pivaloylpiperidin-4-yl)-4-fluorobenzamide (305 mg) was obtained.

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.20 (9H, s), 1.41 (2H, m), 1.7-1.9 (2H, m), 2.91 (2H, br t,  $J=11.9\text{Hz}$ ), 4.07 (1H, m), 4.27 (2H, br d,  $J=13.3\text{Hz}$ ), 7.29 (2H, t,  $J=8.9\text{Hz}$ ), 7.92 (2H, dd,  $J=5.5, 8.9\text{Hz}$ ), 8.30 (1H, d,  $J=7.8\text{Hz}$ )

10 MASS (APCI)(m/z): 329

Reference example 19

To a suspension of N-(piperidin-4-yl)-4-fluorobenzamide (556 mg) in dichloromethane (6 ml) were added cyclopropanecarboxylic acid (0.20 ml), 1-hydroxybenzotriazole (338 mg) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (480 mg) at ambient temperature. After stirring for 21 hours, the mixture was diluted with dichloromethane, and washed with water, saturated aqueous sodium hydrogen carbonate, and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. After crystallization from diisopropyl ether, N-(1-cyclopropylcarbonylpiperidin-4-yl)-4-fluorobenzamide (627 mg) was obtained.

25 NMR (DMSO- $d_6$ ,  $\delta$ ): 0.6-0.8 (4H, m), 1.2-1.6 (2H, m), 1.7-2.0 (2H, m), 1.85 (1H, m), 2.72 (1H, m), 3.21 (1H, m), 4.04 (1H, m), 4.30 (2H, m), 7.29 (2H, t,  $J=8.9\text{Hz}$ ), 7.92 (2H, dd,  $J=5.6, 8.9\text{Hz}$ ), 8.31 (1H, d,  $J=7.7\text{Hz}$ )

MASS (APCI)(m/z): 313

Reference example 20

1-tert-Butoxycarbonyl-4-(4-fluorophenylcarbamoyl)piperazine (0.30 g) was dissolved in a solution of hydrogen chloride in ethyl acetate (4N, 2 ml), and the solution was stirred at ambient temperature for 1 hour. The solvent was removed by evaporation under reduced pressure to give 1-(4-fluorophenylcarbamoyl)piperazine as a white powder, which was taken up into dichloromethane (3 ml), and to the mixture were added in turn pyridine (0.25 ml), 4-trifluoromethoxybenzoyl chloride (0.146 ml), and catalytic amount of N,N-dimethylaminopyridine. After stirring at ambient temperature for 12 hours, the mixture was washed in turn with hydrochloric acid (0.5N), aqueous sodium hydrogen carbonate, and brine, dried over magnesium

sulfate, and evaporated under reduced pressure. The residue was chromatographed on silica gel (50 ml) eluting with 0%-3% methanol in dichloromethane to give 1-(4-fluorophenylcarbamoyl)-4-(4-trifluoromethoxybenzoyl)piperazine (0.19 g).

5 NMR (DMSO- $d_6$ ,  $\delta$ ): 3.2-3.8 (8H, m), 7.08 (2H, t, J=9Hz), 7.35-7.5 (4H, m), 7.5-7.65 (2H, m)

MASS (LD)(m/z): 434.1

#### Reference example 21

10 The following compound was obtained by using methyl chloroformate as a reactive derivative at the carboxy group according to a similar manner to that of Reference example 20.

1-Methoxycarbonyl-4-(4-fluorophenylcarbamoyl)piperazine

NMR (DMSO- $d_6$ ,  $\delta$ ): 3.3-3.5 (8H, m), 3.62 (3H, s), 7.07 (2H, t, J=9Hz), 7.44 (2H, dd, J=5, 9Hz), 8.62 (1H, s)

15 MASS (APCI)(m/z): 282

#### Reference example 22

20 A mixture of N-acetylpiperidine-4-carboxylic acid (514 mg), 1-hydroxybenzotriazole (405 mg), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (575 mg) and 4-fluoroaniline (284.2 ml) in dichloromethane (5 ml) was stirred for 18 hours at ambient temperature. The mixture was diluted with dichloromethane and washed with water, saturated aqueous sodium hydrogen carbonate, water, and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 25 40 ml, dichloromethane:methanol = 15:1). After trituration with diisopropyl ether, 1-acetyl-4-(4-fluorophenyl)carbamoylpiperidine (532 mg) was obtained.

30 NMR (DMSO- $d_6$ ,  $\delta$ ): 1.3-1.7 (2H, m), 1.8 (2H, m), 2.01 (3H, s), 2.5 (2H, m), 3.05 (1H, br t, J=10.6Hz), 3.87 (1H, br d, J=14.1Hz), 4.40 (1H, br d, J=13.1Hz), 7.12 (2H, t, J=8.9Hz), 7.61 (2H, dd, J=5.1, 9.1Hz), 9.96 (1H, s)

MASS (APCI)(m/z): 265

#### Reference example 23

35 A solution of 1-acetylpiperazine-4-sulfonyl chloride (0.91 g) in chloroform (10 ml) were added 4-fluoroaniline (0.38 ml) and triethylamine (0.56 ml) at ambient temperature. After stirring for 6 days, the solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 100 ml,

dichloromethane:methanol=19:1). After rinse with diisopropyl ether, 1-acetyl-4-(4-fluorophenyl)sulfamoylpiperazine (716 mg) was obtained.

5 NMR (CDCl<sub>3</sub>,  $\delta$ ): 1.97 (3H, s), 3.09 (4H, m), 3.37 (4H, m), 7.20 (4H, m), 10.00 (1H, s)

MASS (APCI)(m/z): 302

Reference example 24

To a solution of O-tert-butyl (1-acetylpiperidin-4-yl)carbamate (0.97 g) in N,N-dimethylformamide (10 ml) was added 60% sodium hydride (0.18 g) at ambient temperature. After stirring for 40 minutes, 4-fluorobenzyl bromide (0.6 ml) was added to the reaction mixture. After additional stirring for 4 hours, the reaction mixture was poured into a mixture of ethyl acetate (50 ml) and water (10 ml). An organic phase was separated and washed with water and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 100 ml, toluene:ethyl acetate = 1:1 to 1:2). After crystallization from diisopropyl ether and n-hexane, O-tert-butyl N-(4-fluorobenzyl)-N-(1-acetylpiperidin-4-yl)carbamate (922 mg) was obtained.

20 NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.35 (9H, br s), 1.3-1.8 (4H, m), 1.95 (3H, s), 2.3-2.6 (1H, m), 2.97 (1H, m), 3.80 (1H, br d, J=15.2Hz), 4.0 (1H, m), 4.32 (2H, s), 4.2-4.6 (1H, m), 7.0-7.4 (4H, m)

MASS (APCI)(m/z): 295

Reference example 25

25 To a solution of O-tert-butyl N-(4-fluorobenzyl)-N-(1-acetylpiperidin-4-yl)carbamate (0.5 g) in dichloromethane (5 ml) was added 4N hydrogen chloride in dioxane (5 ml). The reaction mixture was diluted with diisopropyl ether and the precipitates were collected by filtration. After drying under reduced pressure, 1-acetyl-4-(4-fluorobenzyl)-aminopiperidine hydrochloride (409 mg) was obtained.

30 NMR (DMSO-d<sub>6</sub>+D<sub>2</sub>O,  $\delta$ ): 1.54 (2H, m), 2.02 (3H, s), 2.0-2.3 (2H, m), 2.4-2.7 (1H, m), 3.04 (1H, br t, J=12.1Hz), 3.29 (1H, m), 3.9 (1H, m), 4.17 (2H, s), 4.44 (1H, br d, J=13.6Hz), 7.27 (2H, t, J=8.9Hz), 7.66 (2H, br t, J=6.8Hz)

35 MASS (APCI)(m/z): 251

Reference example 26

To a solution of N-(1-acetylpiperidin-4-yl)-4-fluorobenzamide (529 mg) in N,N-dimethylformamide (5 ml) was added sodium hydride (0.1

g). After stirring for 45 minutes, methyl iodide (623 ml) was added to the solution. After stirring for 45 minutes, the mixture was diluted with ethyl acetate (100 ml) and water (50 ml). An organic phase was separated, and washed with water and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. After trituration with diisopropyl ether, N-(1-acetylpiperidin-4-yl)-N-methyl-4-fluorobenzamide (248 mg) was obtained.

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.65 (4H, m), 2.00 (3H, s), 2.78 (3H, s), 3.8 (1H, m), 4.4 (1H, m), 2.0-4.6 (3H, br m), 7.26 (2H, t, J=8.9Hz), 7.46 (2H, dd, J=5.6, 8.7Hz)

MASS (APCI)(m/z): 301

#### Reference example 27

A suspension of 1-acetylpiperazine (0.627 g), 2-chloro-4'-fluoroacetophenone (0.844 g), and potassium hydrogen carbonate (0.735 g) in acetonitrile (12 ml) was stirred at ambient temperature for 3 days. After removal of the solid by filtration, the filtrate was evaporated under reduced pressure to give a residue, which was chromatographed on silica gel (100 ml) eluting with 0%-5% methanol in dichloromethane. The objective compound of the free form was taken up into ethyl acetate (2 ml) and to the solution was added a solution of hydrogen chloride in ethyl acetate (4N, 2 ml). The resulting precipitate was collected by filtration, washed with diisopropyl ether, and dried in vacuo to give 1-acetyl-4-(4-fluorophenylcarbonylmethyl)-piperazine hydrochloride (1.47 g).

NMR (DMSO- $d_6$ ,  $\delta$ ): 2.06 (3H, s), 2.95-3.8 (6H, m), 3.9-4.15 (1H, m), 4.2-4.45 (1H, m), 5.13 (2H, s), 7.48 (2H, t, J=9Hz), 8.09 (2H, dd, J=5, 9Hz)

MASS (APCI)(m/z): 265

30

#### **Industrial Applicability**

An agent for expression of long-term potentiation of synaptic transmission comprising a compound having a brain somatostatin activation property of the present invention is effective for the prophylaxis and/or treatment of cerebral diseases such as dementia (e.g., senile dementia, Alzheimer's dementia, dementia associated with various diseases such as cerebral vascular dementia, cerebral post-traumatic dementia, dementia due to brain tumor, dementia due to chronic subdural hematoma, dementia due to normal pressure



hydrocephalus, post-meningitis dementia, Parkinson's disease type dementia, and the like), amnesia, manic-depressive psychosis, schizophrenia, Parkinson's disease, psychosomatic disease, and the like, particularly for the prophylaxis and/or treatment of dementia  
5 and amnesia. The present invention also relates to a screening method of an agent for the expression of long-term potentiation of synaptic transmission, which uses a somatostatin releasing property as an index. By using the present screening method, various compounds useful for the prophylaxis and/or treatment of the above-mentioned cerebral  
10 diseases can be selected.

This invention is based on application No. 09/321,745 filed in the United States of America, the content of which is incorporated hereinto by reference.

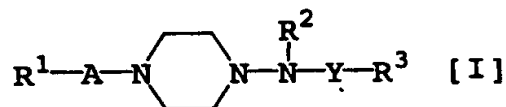
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## CLAIMS

1. An agent for expression of long-term potentiation of synaptic transmission, which comprises a compound having a brain somatostatin  
5 activation property as an active ingredient.

2. The agent for expression of long-term potentiation of synaptic transmission of claim 1, wherein the compound exerts an action to promote  
10 a release of brain somatostatin through suppression of a negative feedback mechanism of brain somatostatin release.

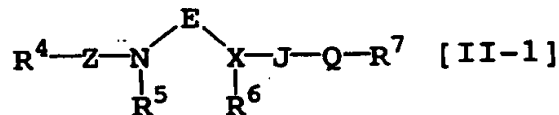
3. The agent for expression of long-term potentiation of synaptic transmission of claim 1 or claim 2, wherein the compound has the following  
15 formula [I]:



wherein

- $\text{R}^1$  is lower alkyl, aryl, ar(lower)alkoxy or heterocyclic group, each of which may be substituted with halogen,  
20  $\text{R}^2$  is hydrogen atom or lower alkyl,  
 $\text{R}^3$  is cyclo(lower)alkyl, aryl or ar(lower)alkyl, each of which may be substituted with halogen,  
 $\text{A}$  is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or lower alkylene, and  
 $\text{Y}$  is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or  $\text{---CONH---}$ ,  
25 or pharmaceutically acceptable salts thereof.

4. The agent for expression of long-term potentiation of synaptic transmission of claim 1 or claim 2, wherein the compound has the following  
30 formula [II-1]:



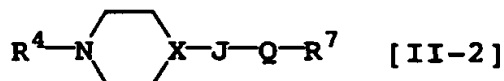
wherein

- $R^4$  is acyl,  
 $R^7$  is lower alkyl, lower alkoxy, lower alkylamino, lower alkenyl, lower alkenyloxy, lower alkenylamino, lower alkynyl, lower alkynyloxy, lower alkynylamino,  
 5 cyclo(lower)alkyl, cyclo(lower)alkyloxy, cyclo(lower)alkylamino, aryl, aryloxy, arylamino, a heterocyclic group or amino substituted with a heterocyclic group, each of which may be substituted with suitable substituent(s); or acyl;  
 10 Z is a single bond, -CO- or -SO<sub>2</sub>-,  
 E is lower alkylene optionally substituted with suitable substituent(s),  
 X is CH or N,  
 J is a single bond, lower alkylene or  
 15



- wherein  $R^8$  is hydrogen, lower alkyl, substituted-lower alkyl, an N-protective group, aryl, acyl or a heterocyclic group,  
 Q is -CH<sub>2</sub>-, -CO-, -SO<sub>2</sub>- or -N=CH-, and  
 20  $R^5$  and  $R^6$  are each hydrogen or lower alkyl, or are taken together to form lower alkylene optionally condensed with a cyclic hydrocarbon or a heterocyclic ring,  
 provided that when X is N,  
 then 1) J is a single bond, and Q is -CH<sub>2</sub>-, -CO- or -SO<sub>2</sub>-, or  
 25 2) J is lower alkylene,  
 or pharmaceutically acceptable salts thereof.

5. The agent for expression of long-term potentiation of synaptic transmission of claim 1 or claim 2, wherein the compound has the following  
 30 formula [II-2]:



wherein

- $R^4$  is acyl,  
 $R^7$  is aryl, aryloxy or arylamino, the aryl moiety of all of which may be substituted with halogen; pyridyl; or pyridylamino;  
 5     $X$  is CH or N,  
        $J$  is a single bond, lower alkylene or



- wherein  $R^8$  is hydrogen, lower alkyl or an N-protective group,  
 10     $Q$  is  $-CH_2-$ ,  $-CO-$  or  $-SO_2-$ ,  
 provided that when  $X$  is N, then  $J$  is a single bond or lower alkylene, or pharmaceutically acceptable salts thereof.

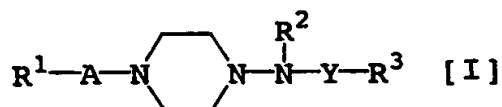
6. The agent for expression of long-term potentiation of synaptic transmission of any of claim 1 to claim 5, which is an agent for the prophylaxis or treatment of cerebral diseases.

7. The agent for expression of long-term potentiation of synaptic transmission of claim 6, which is an agent for the prophylaxis or treatment of dementia or amnesia.

8. A method for expressing long-term potentiation of synaptic transmission, comprising administering an effective amount of a compound having a brain somatostatin activation property.

9. The method for expressing long-term potentiation of synaptic transmission of claim 8, wherein the compound exerts an action to promote a release of brain somatostatin through suppression of a negative feedback mechanism of brain somatostatin release.

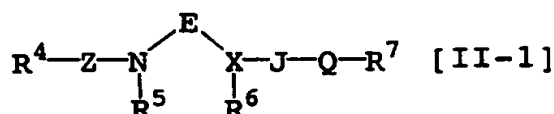
10. The method for expressing long-term potentiation of synaptic transmission of claim 8 or claim 9, wherein the compound has the following formula [I]:



wherein

- 5  $\text{R}^1$  is lower alkyl, aryl, ar(lower)alkoxy or heterocyclic group, each of which may be substituted with halogen,  
 $\text{R}^2$  is hydrogen atom or lower alkyl,  
 $\text{R}^3$  is cyclo(lower)alkyl, aryl or ar(lower)alkyl, each of which may be substituted with halogen,  
 10  $\text{A}$  is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or lower alkylene, and  
 $\text{Y}$  is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or  $\text{---CONH---}$ ,  
 or pharmaceutically acceptable salts thereof.

11. The method for expressing long-term potentiation of synaptic transmission of claim 8 or claim 9, wherein the compound has the following  
 15 formula [II-1]:



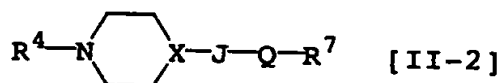
wherein

- 20  $\text{R}^4$  is acyl,  
 $\text{R}^7$  is lower alkyl, lower alkoxy, lower alkylamino, lower alkenyl, lower alkenyloxy, lower alkenylamino, lower alkynyl, lower alkynyloxy, lower alkynylamino, cyclo(lower)alkyl, cyclo(lower)alkyloxy, cyclo(lower)alkylamino, aryl, aryloxy, arylamino,  
 25 a heterocyclic group or amino substituted with a heterocyclic group, each of which may be substituted with suitable substituent(s); or acyl;  
 $\text{Z}$  is a single bond,  $\text{---CO---}$  or  $\text{---SO}_2\text{---}$ ,  
 $\text{E}$  is lower alkylene optionally substituted with suitable substituent(s),  
 30  $\text{X}$  is CH or N,  
 $\text{J}$  is a single bond, lower alkylene or



- wherein  $R^8$  is hydrogen, lower alkyl, substituted-lower alkyl, an N-protective group, aryl, acyl or a heterocyclic group, is  $-CH_2-$ ,  $-CO-$ ,  $-SO_2-$  or  $-N=CH-$ , and  $R^5$  and  $R^6$  are each hydrogen or lower alkyl, or are taken together to form lower alkylene optionally condensed with a cyclic hydrocarbon or a heterocyclic ring, provided that when X is N, then 1) J is a single bond, and Q is  $-CH_2-$ ,  $-CO-$  or  $-SO_2-$ , or 2) J is lower alkylene, or pharmaceutically acceptable salts thereof.

12. The method for expressing long-term potentiation of synaptic transmission of claim 8 or claim 9, wherein the compound has the following formula [II-2]:



- wherein
- 20  $R^4$  is acyl,  
 $R^7$  is aryl, aryloxy or arylamino, the aryl moiety of all of which may be substituted with halogen; pyridyl; or pyridylamino;  
 X is CH or N,  
 25 J is a single bond, lower alkylene or



- wherein  $R^8$  is hydrogen, lower alkyl or an N-protective group, Q is  $-CH_2-$ ,  $-CO-$  or  $-SO_2-$ ,  
 30 provided that when X is N, then J is a single bond or lower alkylene,

or pharmaceutically acceptable salts thereof.

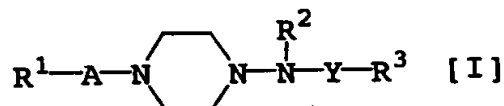
13. The method for expressing long-term potentiation of synaptic transmission of any of claim 8 to claim 12, which is a method for the prophylaxis or treatment of cerebral diseases.

14. The method for expressing long-term potentiation of synaptic transmission of claim 13, which is a method for the prophylaxis and/or treatment of dementia or amnesia.

15. Use of a compound having a brain somatostatin activation property for the production of an agent for the expression of long-term potentiation of synaptic transmission.

16. The use of a compound having a brain somatostatin activation property according to claim 15, wherein the compound exerts an action to promote a release of brain somatostatin through suppression of a negative feedback mechanism of brain somatostatin release.

17. The use of a compound having a brain somatostatin activation property according to claim 15 or claim 16, wherein the compound has the following formula [I]:



wherein

$\text{R}^1$  is lower alkyl, aryl, ar(lower)alkoxy or heterocyclic group, each of which may be substituted with halogen,

$\text{R}^2$  is hydrogen atom or lower alkyl,

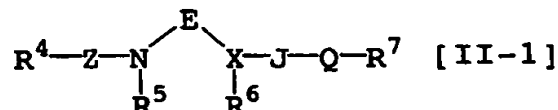
$\text{R}^3$  is cyclo(lower)alkyl, aryl or ar(lower)alkyl, each of which may be substituted with halogen,

A is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or lower alkylene, and

Y is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or  $\text{---CONH---}$ ,

or pharmaceutically acceptable salts thereof.

18. The use of a compound having a brain somatostatin activation property according to claim 15 or claim 16, wherein the compound has the following formula [II-1]:



5

wherein

$\text{R}^4$  is acyl,

$\text{R}^7$  is lower alkyl, lower alkoxy, lower alkylamino, lower alkenyl, lower alkenyloxy, lower alkenylamino, lower alkynyl, lower alkynyloxy, lower alkynylamino, cyclo(lower)alkyl, cyclo(lower)alkyloxy,

10

cyclo(lower)alkylamino, aryl, aryloxy, arylamino, a heterocyclic group or amino substituted with a heterocyclic group, each of which may be substituted with suitable substituent(s); or acyl;

15

$\text{Z}$  is a single bond,  $-\text{CO}-$  or  $-\text{SO}_2-$ ,

$\text{E}$  is lower alkylene optionally substituted with suitable substituent(s),

$\text{X}$  is CH or N,

20

$\text{J}$  is a single bond, lower alkylene or



wherein  $\text{R}^8$  is hydrogen, lower alkyl, substituted-lower alkyl, an N-protective group, aryl, acyl or a heterocyclic group, is  $-\text{CH}_2-$ ,  $-\text{CO}-$ ,  $-\text{SO}_2-$  or  $-\text{N}=\text{CH}-$ , and  $\text{R}^5$  and  $\text{R}^6$  are each hydrogen or lower alkyl, or are taken together to form lower alkylene optionally condensed with a cyclic hydrocarbon or a heterocyclic ring,

25

$\text{Q}$

is  $-\text{CH}_2-$ ,  $-\text{CO}-$ ,  $-\text{SO}_2-$  or  $-\text{N}=\text{CH}-$ , and  $\text{R}^5$  and  $\text{R}^6$  are each hydrogen or lower alkyl, or are taken together to form lower alkylene optionally condensed with a cyclic hydrocarbon or a heterocyclic ring,

provided that when  $\text{X}$  is N,

30

then 1)  $\text{J}$  is a single bond, and  $\text{Q}$  is  $-\text{CH}_2-$ ,  $-\text{CO}-$  or  $-\text{SO}_2-$ , or

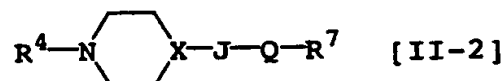
2)  $\text{J}$  is lower alkylene,

or pharmaceutically acceptable salts thereof.



19. The use of a compound having a brain somatostatin activation property according to claim 15 or claim 16, wherein the compound has the following formula [II-2]:

5



wherein

- 10  $R^4$  is acyl,  
 $R^7$  is aryl, aryloxy or arylamino, the aryl moiety of all of which may be substituted with halogen; pyridyl; or pyridylamino;  
 $X$  is CH or N,  
 $J$  is a single bond, lower alkylene or



15

- wherein  $R^8$  is hydrogen, lower alkyl or an N-protective group,  
 $Q$  is  $-CH_2-$ ,  $-CO-$  or  $-SO_2-$ ,  
 provided that when  $X$  is N, then  $J$  is a single bond or lower alkylene, or pharmaceutically acceptable salts thereof.

20

20. The use of a compound having a brain somatostatin activation property according to any of claim 15 to claim 19, which is for the production of an agent for the prophylaxis and/or treatment of cerebral diseases.

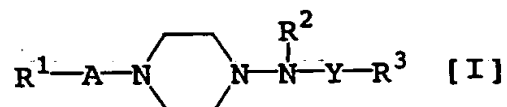
- 25 21. The use of a compound having a brain somatostatin activation property according to claim 20, which is for the production of an agent for the prophylaxis and/or treatment of dementia or amnesia.

- 30 22. A pharmaceutical composition for expression of long-term potentiation of synaptic transmission, which comprises a compound having a brain somatostatin activation property, and a pharmaceutically acceptable carrier or excipient.

23. The pharmaceutical composition for expression of long-term potentiation of synaptic transmission of claim 22, wherein the compound exerts an action to promote a release of brain somatostatin through suppression of a negative feedback mechanism of brain somatostatin release.

24. The pharmaceutical composition for expression of long-term potentiation of synaptic transmission of claim 22 or claim 23, wherein the compound has the following formula [I]:

10

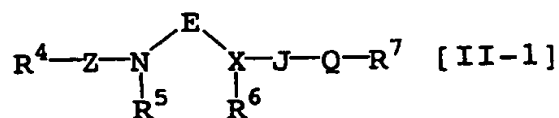


wherein

- $\text{R}^1$  is lower alkyl, aryl, ar(lower)alkoxy or heterocyclic group, each of which may be substituted with halogen,
- 15  $\text{R}^2$  is hydrogen atom or lower alkyl,
- $\text{R}^3$  is cyclo(lower)alkyl, aryl or ar(lower)alkyl, each of which may be substituted with halogen,
- $\text{A}$  is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or lower alkylene, and
- $\text{Y}$  is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or  $\text{---CONH---}$ ,
- 20 or pharmaceutically acceptable salts thereof.

25. The pharmaceutical composition for expression of long-term potentiation of synaptic transmission of claim 22 or claim 23, wherein the compound has the following formula [II-1]:

25



wherein

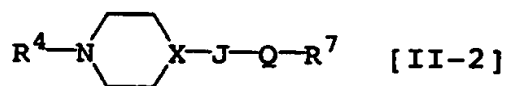
- $\text{R}^4$  is acyl,
- 30  $\text{R}^7$  is lower alkyl, lower alkoxy, lower alkylamino, lower alkenyl, lower alkenyloxy, lower alkenylamino, lower alkynyl, lower alkynyloxy, lower alkynylamino,

- cyclo(lower)alkyl, cyclo(lower)alkyloxy,  
 cyclo(lower)alkylamino, aryl, aryloxy, arylamino,  
 a heterocyclic group or amino substituted with  
 a heterocyclic group, each of which may be substituted with  
 5 suitable substituent(s); or acyl;  
 Z is a single bond, -CO- or -SO<sub>2</sub>-,  
 E is lower alkylene optionally substituted with suitable  
 substituent(s),  
 X is CH or N,  
 10 J is a single bond, lower alkylene or



- wherein R<sup>8</sup> is hydrogen, lower alkyl, substituted-lower alkyl,  
 an N-protective group, aryl, acyl or a heterocyclic group,  
 15 Q is -CH<sub>2</sub>-, -CO-, -SO<sub>2</sub>- or -N=CH-, and  
 R<sup>5</sup> and R<sup>6</sup> are each hydrogen or lower alkyl, or are taken together  
 to form lower alkylene optionally condensed with a cyclic  
 hydrocarbon or a heterocyclic ring,  
 provided that when X is N,  
 20 then 1) J is a single bond, and Q is -CH<sub>2</sub>-, -CO- or -SO<sub>2</sub>-, or  
 2) J is lower alkylene,  
 or pharmaceutically acceptable salts thereof.

26. The pharmaceutical composition for expression of long-term  
 25 potentiation of synaptic transmission of claim 22 or claim 23, wherein  
 the compound has the following formula [II-2]:



- wherein  
 30 R<sup>4</sup> is acyl,  
 R<sup>7</sup> is aryl, aryloxy or arylamino, the aryl moiety of all of  
 which may be substituted with halogen; pyridyl; or  
 pyridylamino;

X is CH or N,  
J is a single bond, lower alkylene or



- 5                    wherein  $R^8$  is hydrogen, lower alkyl or an N-protective group,  
Q                    is  $-CH_2-$ ,  $-CO-$  or  $-SO_2-$ ,  
provided that when X is N, then J is a single bond or lower alkylene,  
or pharmaceutically acceptable salts thereof.
- 10    27. The pharmaceutical composition for expression of long-term  
potentiation of synaptic transmission of any of claim 22 to claim 26,  
which is a pharmaceutical composition for the prophylaxis or treatment  
of cerebral diseases.
- 15    28. The pharmaceutical composition for expression of long-term  
potentiation of synaptic transmission of claim 27, which is a  
pharmaceutical composition for the prophylaxis or treatment of dementia  
or amnesia.
- 20    29. A method for screening an agent for expression of long-term  
potentiation of synaptic transmission, which comprises using a  
somatostatin releasing action as an index.
- 25    30. The screening method of claim 29, which is a screening method of  
an anti-dementia agent or anti-amnesia agent.
- 30    31. A method for screening an agent for expression of long-term  
potentiation of synaptic transmission, which comprises stimulating  
hippocampal slices, bringing a hippocampal slice into contact with  
a test compound, measuring an amount of somatostatin released from  
the hippocampal slice and/or a release time thereof, measuring an amount  
of somatostatin released from a hippocampal slice and/or a release  
time thereof in the absence of a contact with the test compound, and  
comparing the amounts and/or the times to calculate the amount of  
35    somatostatin released from the hippocampal slice and/or the release

FIG.1

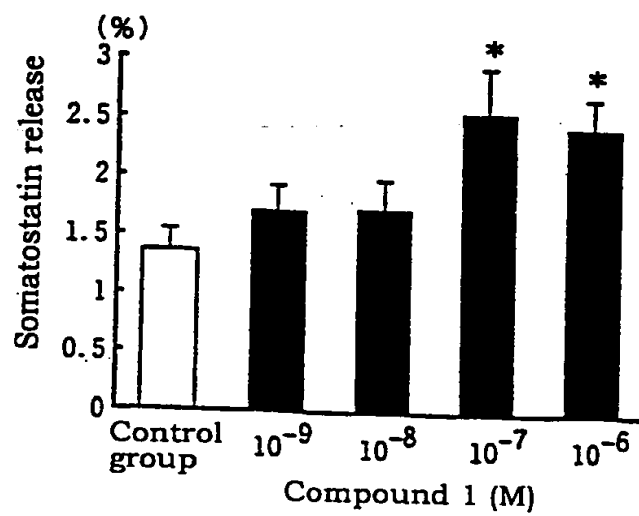


FIG.2

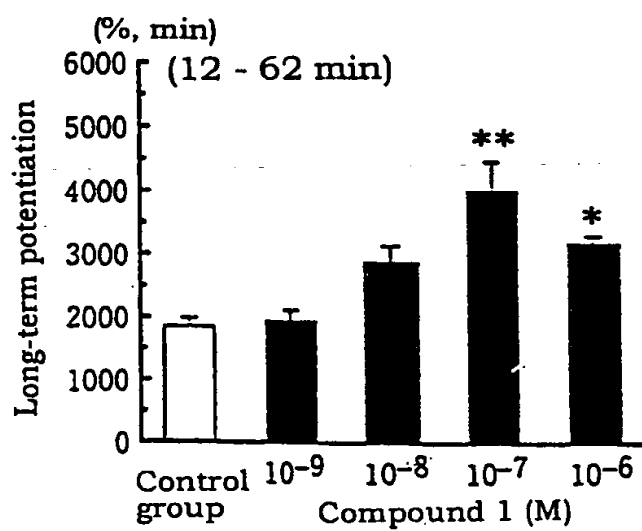


FIG.3

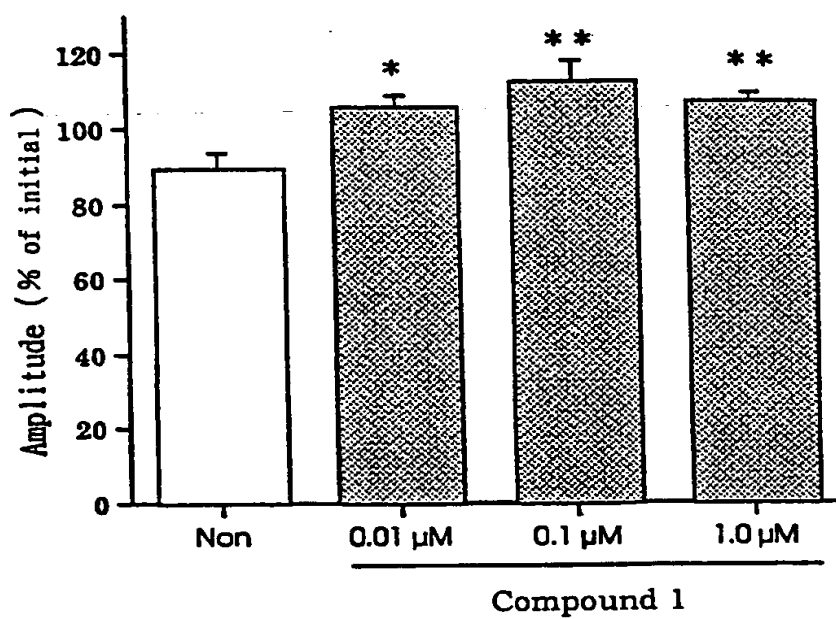
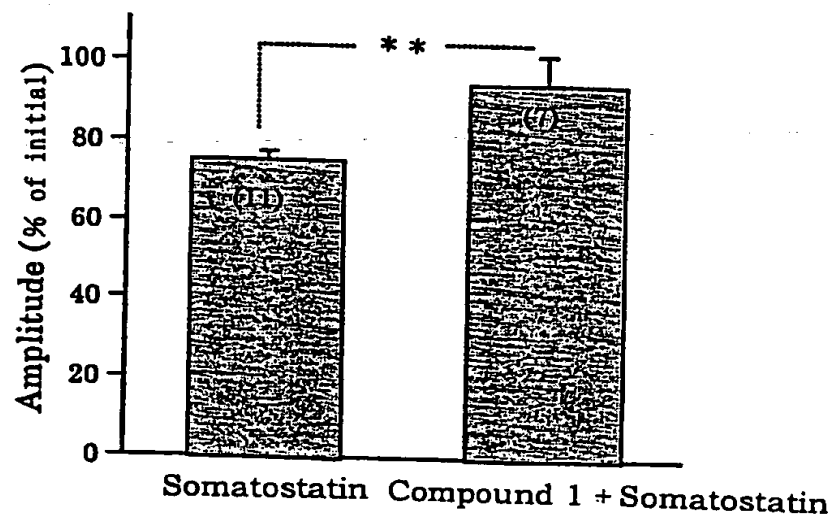


FIG.4





CORRECTED VERSION

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International Bureau



(43) International Publication Date  
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- (74) Agent: **TAKASHIMA, Hajime**; Fujimura Yamato Seimei Bldg., 2-14, Fushimimachi 4-chome, Chuo-ku, Osaka-shi, Osaka 541-0044 (JP).
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see PCT Gazette No. 51/2001 of 20 December 2001, Section II
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: **AGENT FOR EXPRESSION OF LONG-TERM POTENTIATION OF SYNAPTIC TRANSMISSION COMPRISING COMPOUND HAVING BRAIN SOMATOSTATIN ACTIVATION PROPERTY**

(57) Abstract: The present invention relates to an agent for the expression of long-term potentiation of synaptic transmission, which contains a compound having a brain somatostatin activation property as an active ingredient and to a screening method of an agent for the expression of long-term potentiation of synaptic transmission, which uses a somatostatin releasing property as an index. The present invention is useful for the prophylaxis and/or treatment of cerebral diseases of dementia, amnesia, manic-depressive psychosis, schizophrenia, Parkinson's disease, psychosomatic disease and the like.

WO 00/72834 A2

### DESCRIPTION

**AGENT FOR EXPRESSION OF LONG-TERM  
POTENTIATION OF SYNAPTIC TRANSMISSION COMPRISING  
COMPOUND HAVING BRAIN SOMATOSTATIN ACTIVATION PROPERTY**

**5** **Technical Field**

The present invention relates to an agent for the expression of long-term potentiation of synaptic transmission, an anti-dementia agent and an anti-amnesia agent, all of which containing a compound having a brain somatostatin activation property. More particularly, the present invention relates to an agent for the expression of long-term potentiation of synaptic transmission, an anti-dementia agent and an anti-amnesia agent, all of which containing a compound exerting a brain somatostatin release promoting action through suppression of the negative feedback mechanism of brain somatostatin release. The present invention moreover relates to a method for expressing long-term potentiation of synaptic transmission, a method for the treatment and/or prophylaxis of dementia and amnesia, and a screening method of these drugs using a somatostatin releasing property as an index.

## Background Art

20       The hippocampal function is said to be responsible for learning and memory. When an input neuron of the hippocampus is stimulated for a short time at high frequency, the efficiency of synaptic transmission continues to increase for a long time thereafter. This phenomenon is called long-term potentiation (hereinafter also referred to as LTP) 25 of synaptic transmission, and has been recognized as a cellular model of learning and memory (T. V. P. Bliss and G. L. Collingridge, Nature vol. 361, p. 31, 1993). There is a demand for further elucidation of the mechanism of the LTP and the relation thereof with learning and memory. Also, a search for a compound having a property of long-term 30 potentiation of synaptic transmission has been desired.

Somatostatin has been known for quite a long time as a hypothalamic hormone capable of suppressing the secretion of somatotropin from the pituitary gland. It has been recently found that it is also present in the cerebral cortex and the hippocampus, that are important cerebral sites for memory and learning, at high concentrations, playing an important role in memory and learning as a neuromodulator.

## Disclosure of the Invention

As a result of the intensive studies of the present inventors,

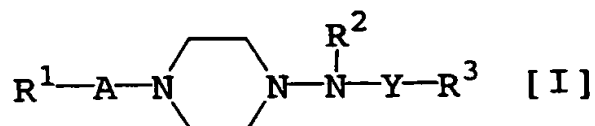
it has been newly found that a compound having a brain somatostatin release promotion property expresses long-term potentiation of synaptic transmission. Based on this new finding, the inventors have found that administration of a compound having a brain somatostatin release promoting property leads to the prophylaxis and/or treatment of cerebral diseases such as dementia, amnesia, manic-depressive psychosis, schizophrenia, Parkinson's disease, psychosomatic disease, and the like, which resulted in the completion of the present invention.

Accordingly, the present invention provides the following.

(1) An agent for expression of long-term potentiation of synaptic transmission, which comprises a compound having a brain somatostatin activation property as an active ingredient;  
a method for expressing long-term potentiation of synaptic transmission, comprising administering an effective amount of a compound having a brain somatostatin activation property;  
use of a compound having a brain somatostatin activation property for the production of an agent for the expression of long-term potentiation of synaptic transmission; and  
a pharmaceutical composition for expression of long-term potentiation of synaptic transmission, which comprises a compound having a brain somatostatin activation property, and a pharmaceutically acceptable carrier or excipient.

(2) The agent, the method, the use and the pharmaceutical composition of (1), wherein the compound exerts an action to promote a release of brain somatostatin through suppression of a negative feedback mechanism of brain somatostatin release.

(3) The agent, the method, the use and the pharmaceutical composition of (1) or (2), wherein the compound has the following formula [I]:



wherein

$\text{R}^1$  is lower alkyl, aryl, ar(lower)alkoxy or heterocyclic group, each of which may be substituted with halogen,

$\text{R}^2$  is hydrogen atom or lower alkyl,

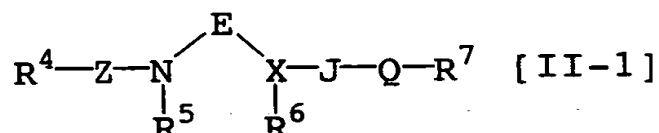
$\text{R}^3$  is cyclo(lower)alkyl, aryl or ar(lower)alkyl, each of which

may be substituted with halogen,  
 A is -CO-, -SO<sub>2</sub>- or lower alkylene, and

Y is -CO-, -SO<sub>2</sub>- or -CONH-,

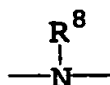
or pharmaceutically acceptable salts thereof.

- 5 (4) The agent, the method, the use and the pharmaceutical composition of (1) or (2), wherein the compound has the following formula [II-1]:



wherein

- 10 R<sup>4</sup> is acyl,  
 R<sup>7</sup> is lower alkyl, lower alkoxy, lower alkylamino, lower alkenyl, lower alkenyloxy, lower alkenylamino, lower alkynyl, lower alkynyloxy, lower alkynylamino, cyclo(lower)alkyl, cyclo(lower)alkyloxy,  
 15 cyclo(lower)alkylamino, aryl, aryloxy, arylamino, a heterocyclic group or amino substituted with a heterocyclic group, each of which may be substituted with suitable substituent(s); or acyl;  
 Z is a single bond, -CO- or -SO<sub>2</sub>-,  
 20 E is lower alkylene optionally substituted with suitable substituent(s),  
 X is CH or N,  
 J is a single bond, lower alkylene or



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wherein R<sup>8</sup> is hydrogen, lower alkyl, substituted-lower alkyl, an N-protective group, aryl, acyl or a heterocyclic group,

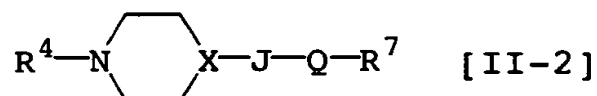
Q is -CH<sub>2</sub>-, -CO-, -SO<sub>2</sub>- or -N=CH-, and

- 30 R<sup>5</sup> and R<sup>6</sup> are each hydrogen or lower alkyl, or are taken together to form lower alkylene optionally condensed with a cyclic hydrocarbon or a heterocyclic ring, provided that when X is N,

then 1) J is a single bond, and Q is -CH<sub>2</sub>-, -CO- or -SO<sub>2</sub>-, or  
 2) J is lower alkylene,

or pharmaceutically acceptable salts thereof.

(5) The agent, the method, the use and the pharmaceutical composition  
 5 of (1) or (2), wherein the compound has the following formula [II-2]:



wherein

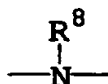
R<sup>4</sup> is acyl,

10 R<sup>7</sup> is aryl, aryloxy or arylamino, the aryl moiety of all of  
 which may be substituted with halogen; pyridyl; or  
 pyridylamino;

X is CH or N,

J is a single bond, lower alkylene or

15



wherein R<sup>8</sup> is hydrogen, lower alkyl or an N-protective group,

Q is -CH<sub>2</sub>-, -CO- or -SO<sub>2</sub>-,

provided that when X is N, then J is a single bond or lower alkylene,  
 20 or pharmaceutically acceptable salts thereof.

(6) The agent, the method and the pharmaceutical composition of any  
 of (1) to (5), which is for the prophylaxis and/or treatment of cerebral  
 diseases; and the use according to any of (1) to (5), which is for  
 the production of an agent for the prophylaxis and/or treatment of  
 25 cerebral diseases.

(7) The agent, the method and the pharmaceutical composition of (6),  
 which is for the prophylaxis and/or treatment of dementia or amnesia;  
 and the use according to (6), which is for the production of an agent  
 for the prophylaxis and/or treatment of dementia or amnesia.

30 (8) A method for screening an agent for expression of long-term  
 potentiation of synaptic transmission, which comprises using a  
 somatostatin releasing action as an index.

(9) The screening method of (8), which is a screening method of an

anti-dementia agent or anti-amnesia agent.

(10) A method for screening an agent for expression of long-term potentiation of synaptic transmission, which comprises stimulating hippocampal slices, bringing a hippocampal slice into contact with a test compound, measuring an amount of somatostatin released from the hippocampal slice and/or a release time thereof, measuring an amount of somatostatin released from a hippocampal slice and/or a release time thereof in the absence of a contact with the test compound, and comparing the amounts and/or the times to calculate the amount of somatostatin released from the hippocampal slice and/or the release time thereof caused by the contact with the test compound.

(11) The screening method according to (10), which is a screening method of an anti-dementia agent or anti-amnesia agent.

(12) The agent, the method, the use and the pharmaceutical composition according to (1), wherein the compound having the brain somatostatin activation property is obtained by the screening method of any of (8) to (11).

(13) A commercial package comprising the pharmaceutical composition for expression of long-term potentiation of synaptic transmission of any of (1) - (7), (12) and a written matter associated therewith, wherein the written matter states that the pharmaceutical composition can or should be used for expression of long-term potentiation of synaptic transmission.

(14) A compound selected by the screening method described in any of (8) to (11).

#### Brief Description of the Drawing

Fig. 1 is a bar graph showing the action of compound 1 to be mentioned later, i.e., N-(4-acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate, on somatostatin release from rat hippocampal slice upon stimulation with 50mM K<sup>+</sup>, wherein the axis of ordinate is somatostatin release (%) and each value is mean  $\pm$  S.E.M (n=10 - 11). The symbol \* means that Dunnett's multiple comparison test showed a significant difference by P<0.05 of the group containing various concentrations of compound 1 as compared to the control group.

Fig. 2 is a bar graph showing the action of compound 1 on hippocampus long-term potentiation phenomenon, wherein the axis of ordinate shows the magnitude of LTP by the integral (%·min) of potential variation (%) from 12 minutes to 62 minutes after tetanic stimulation. The symbol

\* means that one-way analysis of variance and Dunnett's multiple comparison test showed a significant difference by  $P < 0.05$  of the group containing various concentrations of compound 1 as compared to the control group. The symbol \*\* means that one-way analysis of variance and Dunnett's multiple comparison test showed a significant difference by  $P < 0.01$  of the group containing various concentrations of compound 1 as compared to the control group.

Fig. 3 is a bar graph showing the dose-response dependency of compound 1 with regard to voltage-dependent calcium channel, wherein the axis of ordinate shows variation (%) of the maximal value of the membrane potential dependent calcium current to the value before the addition of compound 1, wherein each value is  $\text{mean} \pm \text{S.E.M}$  ( $n=7$ ). The symbol \* means that Dunnett's multiple comparison test showed a significant difference by  $P < 0.05$  of the group containing various concentrations of compound 1 as compared to the control group. The symbol \*\* means that Dunnett's multiple comparison test showed a significant difference by  $P < 0.01$  of the group containing various concentrations of compound 1 as compared to the control group.

Fig. 4 is a bar graph showing the action of somatostatin and compound 1 on the membrane potential dependent calcium current, wherein the axis of ordinate shows variation (%) of the maximal value of the membrane potential dependent calcium current upon addition of somatostatin alone or both compound 1 and somatostatin, to the value before addition of somatostatin ( $10^{-7}$  M) alone or both compound 1 ( $10^{-7}$  M) and somatostatin ( $10^{-7}$  M) and each value is  $\text{mean} \pm \text{S.E.M.}$ , and the numerals in parentheses are the number of times of measurements. The symbol \*\* means that Dunnett's multiple comparison test showed a significant difference by  $P < 0.01$  of the group containing compound 1 and somatostatin as compared to the group containing somatostatin.

#### Detailed Description of the Invention

Respective definitions and specific examples thereof used in the present invention, as well as preferable embodiments thereof are explained in detail in the following.

#### Compound having brain somatostatin activation property

The activation property of brain somatostatin means, for example, an action to promote release of brain somatostatin, an action to increase biosynthesis of somatostatin within nerve cells, an action to activate somatostatin receptors, an action to potentiate expression of

somatostatin property, an action to potentiate somatostatin signal transduction and the like.

The compound to be used in the present invention is one capable of activating brain somatostatin based on at least one of the  
5      aforementioned properties. Particularly, a compound having a brain somatostatin release promoting property is preferably used, which is preferably a compound that shows a brain somatostatin release promoting property through suppression of the negative feedback mechanism of brain somatostatin release.

10      Examples of promotion of release of brain somatostatin include actions of, for example, suppression of a negative feedback mechanism of brain somatostatin release, release of suppression by somatostatin of voltage-dependent calcium channel present in neuron, promotion of the voltage-dependent calcium channel, modification of mutual  
15      intracellular action between G protein and calcium channel, phosphorylation of calcium channel, modification of  $K^+$  channel, influencing kinetic behavior of somatostatin-containing vesicle, and the like, whereby release of somatostatin is promoted.

20      The action to increase biosynthesis of somatostatin in the nerve cells may be, for example, an action to potentiate the expression of somatostatin mRNA, an action to promote protein synthesis from mRNA, or an action to promote cleaving out from the precursor of somatostatin, wherein these actions promote the release of brain somatostatin.

25      The brain somatostatin release promoting property is evaluated by the method to be mentioned later.

30      The action to activate somatostatin receptors may be, for example, the actions to stimulate somatostatin receptors, to suppress desensitization of somatostatin receptors, to suppress intracellular transfer of somatostatin receptors, to increase the number of somatostatin receptors present in the postsynaptic membrane and the like.

35      The action to potentiate the expression of somatostatin property may be, for example, an action to suppress the decomposition of somatostatin, an action to suppress the re-uptake of somatostatin and the like.

    The action to potentiate the signal transmission of somatostatin is exemplified by an action to potentiate G protein, cAMP, protein kinase, protein phosphatase, transcription factor and the like, coupled

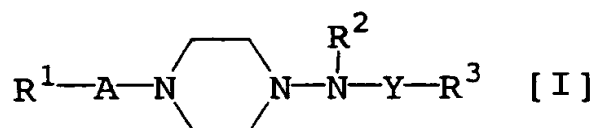


with a somatostatin receptor, wherein the action is directed to an intracellular signal transduction messenger and the like other than somatostatin receptor, thereby to potentiate the signal transduction of somatostatin.

- 5 The compound having a brain somatostatin activation property to be used in the present invention encompasses any compound having such an activation property. Preferable examples thereof include compounds of the following formulas:

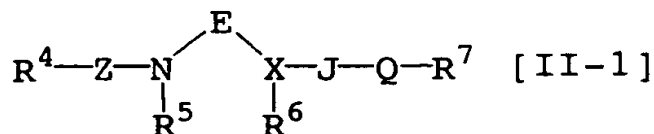
① formula [I]

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wherein

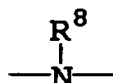
- $\text{R}^1$  is lower alkyl, aryl, ar(lower)alkoxy or heterocyclic group, each of which may be substituted with halogen,  
 15  $\text{R}^2$  is hydrogen atom or lower alkyl,  
 $\text{R}^3$  is cyclo(lower)alkyl, aryl or ar(lower)alkyl, each of which may be substituted with halogen,  
 $\text{A}$  is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or lower alkylene, and  
 $\text{Y}$  is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or  $\text{---CONH---}$   
 20 (EP Publication No. 436734) (hereinafter also referred to as compound [I]), and pharmaceutically acceptable salts thereof and  
 ② formula [II-1]:



25 wherein

- $\text{R}^4$  is acyl,  
 $\text{R}^7$  is lower alkyl, lower alkoxy, lower alkylamino, lower alkenyl, lower alkenyloxy, lower alkenylamino, lower alkynyl, lower alkynyloxy, lower alkynylamino,  
 30 cyclo(lower)alkyl, cyclo(lower)alkyloxy, cyclo(lower)alkylamino, aryl, aryloxy, arylamino, a heterocyclic group or amino substituted with

a heterocyclic group, each of which may be substituted with suitable substituent(s); or acyl;  
 Z is a single bond, -CO- or -SO<sub>2</sub>-,  
 E is lower alkylene optionally substituted with suitable substituent(s),  
 X is CH or N,  
 J is a single bond, lower alkylene or



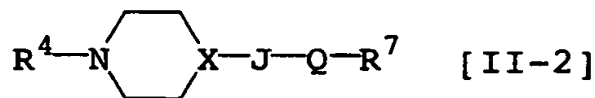
wherein R<sup>8</sup> is hydrogen, lower alkyl, substituted-lower alkyl, an N-protective group, aryl, acyl or a heterocyclic group,  
 Q is -CH<sub>2</sub>-, -CO-, -SO<sub>2</sub>- or -N=CH-, and  
 R<sup>5</sup> and R<sup>6</sup> are each hydrogen or lower alkyl, or are taken together to form lower alkylene optionally condensed with a cyclic hydrocarbon or a heterocyclic ring,  
 provided that when X is N,  
 then 1) J is a single bond, and Q is -CH<sub>2</sub>-, -CO- or -SO<sub>2</sub>-, or  
 2) J is lower alkylene,  
 (hereinafter also referred to as compound [II-1]) and pharmaceutically acceptable salts thereof.

Preferred compound [I] is one which has a lower alkyl, phenyl, naphthyl or thienyl for R<sup>1</sup>, hydrogen or lower alkyl for R<sup>2</sup>, phenyl which may be substituted with a halogen for R<sup>3</sup>, -CO- for A, and -CO- or -SO<sub>2</sub>- for Y.

More preferred compound [I] is one which has a lower alkyl for R<sup>1</sup>, hydrogen for R<sup>2</sup>, phenyl which is substituted with a halogen for R<sup>3</sup>, -CO- for A, and -CO- for Y.

Most preferred compound [I] is N-(4-acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate (compound 1) (International Publication No. WO98/25914).

When Z is a single bond, E is ethylene, and R<sup>5</sup> and R<sup>6</sup> are taken together to form ethylene, preferable compounds [II-1] can be represented by the following general formula [II-2]:



wherein

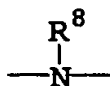
$R^4$  is acyl,

5  $R^7$  is aryl, aryloxy or arylamino, the aryl moiety of all of which may be substituted with halogen; pyridyl; or pyridylamino;

$X$  is CH or N,

$J$  is a single bond, lower alkylene or

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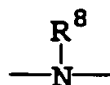


wherein  $R^8$  is hydrogen, lower alkyl or an N-protective group,

$Q$  is  $-CH_2-$ ,  $-CO-$  or  $-SO_2-$ ,

provided that when  $X$  is N, then  $J$  is a single bond or lower alkylene, (hereinafter also referred to as compound [II-2]) and pharmaceutically acceptable salts thereof.

Preferred compound [II-2] is one which has lower alkanoyl, esterified carboxy, substituted or unsubstituted aroyl, lower alkylsulfonyl, substituted or unsubstituted arylsulfonyl, or 20 cyclo(lower)alkylcarbonyl for  $R^4$ , and aryl or arylamino, each aryl of which may be substituted with halogen for  $R^7$ , CH or N for  $X$ , a single bond, lower alkylene or



25 (wherein  $R^8$  is hydrogen, lower alkyl or an N-protective group) for  $J$ , and  $-CH_2-$ ,  $-CO-$  or  $-SO_2-$  for  $Q$ , provided that when  $X$  is N, then  $J$  is a single bond or lower alkylene, and pharmaceutically acceptable salts thereof.

More preferred compound [II-2] is one which has 30 lower alkanoyl, lower alkoxycarbonyl, aroyl, aroyl substituted with

halo(lower)alkoxy, lower alkylsulfonyl, arylsulfonyl, arylsulfonyl substituted with halogen, or cyclo(lower)alkylcarbonyl for R<sup>4</sup>, aryl or arylamino, each aryl of which may be substituted with halogen for R<sup>7</sup>, CH for X, a single bond or -NH- for J, and -CO- or -SO<sub>2</sub>- for Q, and pharmaceutically acceptable salts thereof.

Particularly more preferred compound [II-2] is one which has lower alkanoyl, lower alkoxycarbonyl, aroyl, aroyl substituted with halo(lower)alkoxy, lower alkylsulfonyl, arylsulfonyl, arylsulfonyl substituted with halogen, or cyclo(lower)alkylcarbonyl for R<sup>4</sup>, aryl or arylamino, each aryl of which may be substituted with halogen for R<sup>7</sup>, CH for X, -NH- for J, and -CO- for Q, and pharmaceutically acceptable salts thereof.

Most preferred compound [II-2] is one selected from the group consisting of

N-(1-acetylpiperidin-4-yl)-4-fluorobenzamide,  
N-(1-acetylpiperidin-4-yl)-N'-(4-fluorophenyl)urea,  
4-(4-fluorobenzoylamino)-1-methoxycarbonylpiperidine,  
4-(4-fluorobenzoylamino)-1-(4-fluorophenylsulfonyl)piperidine,  
4-(4-fluorobenzoylamino)-1-(4-trifluoromethoxybenzoyl)piperidine,  
4-(4-fluorobenzoylamino)-1-methylsulfonylpiperidine,  
N-(1-methoxycarbonylpiperidin-4-yl)-N'-(4-fluorophenyl)urea,  
N-(1-(4-fluorophenylsulfonyl)piperidin-4-yl)-N'-(4-fluorophenyl)-urea,  
N-(1-benzoylpiperidin-4-yl)-4-fluorobenzamide,  
N-(1-pivaloylpiperidin-4-yl)-4-fluorobenzamide and  
N-(1-cyclopropylcarbonylpiperidin-4-yl)-4-fluorobenzamide.

In the above and subsequent description of the present specification, suitable examples of the various definitions to be included within the scope of the invention are explained in detail in the following.

The term "lower" is intended to mean a group having 1 to 6 carbon atom(s), unless otherwise provided.

The lower moiety in the terms "lower alkenyl", "lower alkenyloxy", "lower alkenylamino", "lower alkynyl", "lower alkynyloxy" and "lower alkynylamino" is intended to mean a group having 2 to 6 carbon atoms.

The lower moiety in the terms "cyclo(lower)alkyl", "cyclo(lower)alkyloxy" and "cyclo(lower)alkylamino" is intended to mean a group having 3 to 6 carbon atoms.

Suitable "lower alkyl" and lower alkyl moiety in the terms "substituted-lower alkyl", "ar(lower)alkyl", "halo(lower)alkyl", "lower alkylamino", "lower alkylsilyl", "lower alkylthio" and "lower alkylsulfonyl" may be a straight or branched C<sub>1</sub>-C<sub>6</sub> alkyl such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, ethylpropyl, hexyl or the like, in which preferable one is methyl.

Suitable "lower alkenyl" and lower alkenyl moiety in the terms "lower alkenyloxy" and "lower alkenylamino" may be a straight or branched C<sub>2</sub>-C<sub>6</sub> alkenyl such as ethenyl, propenyl, butenyl, pentenyl, hexenyl, isopropenyl, butadienyl, pentadienyl, hexadienyl or the like, in which preferable one is ethenyl, propenyl or butadienyl.

Suitable "lower alkynyl" and lower alkynyl moiety in the terms "lower alkynyloxy" and "lower alkynylamino" may be a straight or branched C<sub>2</sub>-C<sub>6</sub> alkynyl such as ethynyl, propargyl, butynyl or the like, in which preferable one is ethynyl.

Suitable "cyclo(lower)alkyl" and cyclo(lower)alkyl moiety in the terms "cyclo(lower)alkyloxy" and "cyclo(lower)alkylamino" may be cyclo(C<sub>3</sub>-C<sub>6</sub>)alkyl such as cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl, in which preferable one in the definitions of general formulas [II-1] and [II-2] is cyclopropyl.

Suitable "aryl" in the definitions of general formula [I] may be phenyl, naphthyl, tolyl, xylyl, mesityl, cumenyl, and the like, in which preferable one is phenyl or naphthyl.

Suitable "ar(lower)alkoxy" in the definitions of general formula [I] may be benzyloxy, phenethyloxy, phenylpropoxy, benzhydryloxy, trityloxy and the like.

Suitable "aryl" and aryl or ar moiety in the terms "ar(lower)alkoxy", "aryloxy", "arylamino", "arylsulfonyl", "aroyl" and "ar(lower)alkyl" in the definitions of general formulas [II-1] and [II-2] may be phenyl, naphthyl, phenyl substituted with lower alkyl [e.g. tolyl, xylyl, mesityl, cumenyl, di(tert-butyl)phenyl, etc.] and the like, in which preferable one is phenyl or tolyl.

Suitable "ar(lower)alkyl" may be benzyl, phenethyl, phenylpropyl, benzhydryl, trityl and the like, in which preferable one in the definitions of general formulas [II-1] and [II-2] is benzyl.

Suitable "lower alkylene" in the definitions of general formula [I] may be methylene, ethylene, propylene, pentamethylene, hexamethylene, and the like.

Suitable "lower alkylene" and lower alkylene moiety in the term "lower alkylenedioxy" in the definitions of general formulas [II-1] and [II-2] may be a straight or branched C<sub>1</sub>-C<sub>6</sub> alkylene such as methylene, ethylene, trimethylene, propylene, tetramethylene, pentamethylene, hexamethylene, ethylethylene or the like, in which preferable one is methylene, ethylene or trimethylene.

Suitable "lower alkoxy" and lower alkoxy moiety in the terms "ar(lower)alkoxy" and "halo(lower)alkoxy" may be a straight or branched C<sub>1</sub>-C<sub>6</sub> alkoxy such as methoxy, ethoxy, propoxy, isopropoxy, methylpropoxy, butoxy, isobutoxy, tert-butoxy, pentyloxy, hexyloxy or the like, in which preferable one is methoxy or tert-butoxy.

Suitable "heterocyclic group" in the definitions of general formula [I] may include saturated or unsaturated, monocyclic or polycyclic one containing at least one hetero atom such as nitrogen atom, oxygen atom or sulfur atom. The preferred examples of thus defined "heterocyclic group" may be

- unsaturated, 3 to 8-membered, more preferably 5 or 6-membered heteromonocyclic group containing 1 to 4-nitrogen atom(s), for example, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyridyl N-oxide, dihydropyridyl, tetrahydropyridyl, pyrimidyl, pyrazinyl, pyridazinyl, triazinyl, triazolyl, tetrazinyl, tetrazolyl, etc.;
- unsaturated, condensed heterocyclic group containing 1 to 5 nitrogen atom(s), for example, indolyl, isoindolyl, indolizynyl, benzimidazolyl, quinolyl, isoquinolyl, indazolyl, benzotriazolyl, etc.;
- unsaturated, 3 to 8-membered heteromonocyclic group containing 1 to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), for example, oxazolyl, isoxazolyl, oxadiazolyl etc.;
- saturated, 3 to 8-membered heteromonocyclic group containing 1 to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), for example, morpholino, sydnonyl, etc.;
- unsaturated, condensed heterocyclic group containing 1 to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), for example, benzoxazolyl, benzoxadiazolyl, etc.;
- unsaturated, 3 to 8-membered heteromonocyclic group containing 1 to 2 sulfur atom(s) and 1 to 3 nitrogen atom(s), for example, thiazolyl, isothiazolyl, thiadiazolyl etc.;
- unsaturated, 3 to 8-membered heteromonocyclic group containing 1 to 2 sulfur atom(s), for example, thienyl, etc.;

unsaturated, condensed heterocyclic group containing 1 to 2 sulfur atom(s) and 1 to 3 nitrogen atom(s), for example, benzothiazolyl, benzothiadiazolyl, etc.;

5 unsaturated, 3 to 8-membered heteromonocyclic group containing an oxygen atom, for example, furyl, etc.;

unsaturated, condensed heterocyclic group containing 1 to 2 sulfur atom(s), for example, benzothienyl, etc.;

unsaturated, condensed heterocyclic group containing 1 to 2 oxygen atom(s), for example, benzofuranyl, etc.; or the like.

10 The above-mentioned "lower alkyl", "aryl", "ar(lower)alkoxy", "heterocyclic group", "cyclo(lower)alkyl" and "ar(lower)alkyl" in the definitions of general formula [I] may be substituted with halogen [e.g. fluorine, chlorine, bromine and iodine].

Suitable "halogen" and halomoiety in the term "halo(lower)alkyl"  
15 may be fluorine, chlorine, bromine and iodine, in which preferable one is fluorine, chlorine or iodine.

Suitable "halo(lower)alkyl" may be lower alkyl substituted with one or more halogens such as chloromethyl, dichloromethyl, fluoromethyl, difluoromethyl, trifluoromethyl, pentachloroethyl or the like, in which  
20 preferable one is trifluoromethyl.

Suitable "halo(lower)alkoxy" may be lower alkoxy substituted with one or more halogens such as chloromethoxy, dichloromethoxy, fluoromethoxy, difluoromethoxy, trifluoromethoxy, pentachloromethoxy or the like, in which preferable one is trifluoromethoxy.

25 Suitable "lower alkylamino" may be mono or di(lower)alkylamino such as methylamino, ethylamino, propylamino, isopropylamino, butylamino, tert-butylamino, isobutylamino, pentylamino, hexylamino, dimethylamino, diethylamino, dipropylamino, dibutylamino, diisopropylamino, dipentylamino, dihexylamino, N-methylethylamino or  
30 the like, in which preferable one is dimethylamino.

Suitable "lower alkylsilyl" may be mono, di, or tri(lower)alkylsilyl such as trimethylsilyl, dimethylsilyl, triethylsilyl or the like, in which preferable one is trimethylsilyl.

Suitable "lower alkylenedioxy" may be methylenedioxy, ethylenedioxy and the like, in which preferable one is methylenedioxy.  
35

Suitable "heterocyclic group" in the definitions of general formulas [II-1] and [II-2] may be one containing at least one hetero atom selected from nitrogen, sulfur and oxygen atom, and may include

saturated or unsaturated, monocyclic or polycyclic heterocyclic group, and preferable heterocyclic group may be N-containing heterocyclic group such as

unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 4 nitrogen atom(s), for example, pyrrolyl, pyrrolinyl, imidazolyl, pyrazolyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazolyl [e.g. 4H-1,2,4-triazolyl, 1H-1,2,3-triazolyl, 2H-1,2,3-triazolyl, etc.], tetrazolyl [e.g. 1H-tetrazolyl, 2H-tetrazolyl, etc.], etc.;

saturated 3 to 7-membered heteromonocyclic group containing 1 to 4 nitrogen atom(s), [e.g. pyrrolidinyl, imidazolidinyl, piperidyl, piperazinyl, homopiperazinyl, etc.];

unsaturated condensed heterocyclic group containing 1 to 5 nitrogen atom(s), for example, indolyl, isoindolyl, indolizinyl, benzimidazolyl, quinolyl, isoquinolyl, imidazopyridyl, indazolyl, benzotriazolyl, tetrazolopyridazinyl [e.g. tetrazolo[1,5-b]pyridazinyl, etc.], quinoxalinyl, etc.;

unsaturated 3 to 6-membered heteromonocyclic group containing an oxygen atom, for example, pyranyl, furyl, etc.;

saturated 3 to 6-membered heteromonocyclic group containing an oxygen atom, for example, 1H-tetrahydropyranyl, tetrahydrofuranlyl, etc.;

unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 2 sulfur atom(s), for example, thienyl, etc.;

unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), for example, oxazolyl, isoxazolyl, oxadiazolyl [e.g. 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,5-oxadiazolyl, etc.], oxazolinylyl [e.g. 2-oxazolinylyl, etc.], etc.;

saturated 3 to 6-membered heteromonocyclic group containing 1 to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s) [e.g. morpholinyl, etc.];

unsaturated condensed heterocyclic group containing 1 to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s) [e.g. benzofurazanyl, benzoxazolyl, benzoxadiazolyl, etc.];

unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 2 sulfur atom(s) and 1 to 3 nitrogen atom(s), for example, thiazolyl, thiadiazolyl [e.g. 1,2,4-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl, etc.], etc.;

saturated 3 to 6-membered heteromonocyclic group containing 1 to 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) [e.g. thiazolidinyl, etc.];

unsaturated condensed heterocyclic group containing 1 to 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) [e.g. thiazolidinyl, etc.];

unsaturated condensed heterocyclic group containing 1 to 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) [e.g. thiazolidinyl, etc.];

unsaturated condensed heterocyclic group containing 1 to 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) [e.g. thiazolidinyl, etc.];

unsaturated condensed heterocyclic group containing 1 to 2 sulfur atom(s) and 1 to 3 nitrogen atom(s) [e.g. thiazolidinyl, etc.];



atom(s) and 1 to 3 nitrogen atom(s) [e.g. benzothiazolyl, benzothiadiazolyl, etc.]; unsaturated condensed heterocyclic group containing 1 to 2 oxygen atom(s) [e.g. benzofuranyl, benzodioxolyl, chromanyl, etc.] and the like.

Said "heterocyclic group" may be substituted with lower alkyl as exemplified above, in which preferable one is thienyl, pyridyl, methylpyridyl, quinolyl, indolyl, quinoxalinyl, benzofuranyl or tetramethylchromanyl, and more preferable one is pyridyl.

Suitable "acyl" may be carboxy; esterified carboxy; carbamoyl substituted with lower alkyl, aryl, ar(lower)alkyl, arylsulfonyl, lower alkylsulfonyl or a heterocyclic group; substituted or unsubstituted arylsulfonyl; lower alkylsulfonyl; cyclo(lower)alkylcarbonyl; lower alkanoyl; substituted or unsubstituted aroyl; a heterocycliccarbonyl and the like.

The esterified carboxy may be substituted or unsubstituted lower alkoxy carbonyl [e.g. methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl, tert-butoxycarbonyl, hexyloxycarbonyl, 2-iodoethoxycarbonyl, 2,2,2-trichloroethoxycarbonyl, etc.], substituted or unsubstituted aryloxycarbonyl [e.g. phenoxycarbonyl, 4-nitrophenoxycarbonyl, 2-naphthylloxycarbonyl, etc.], substituted or unsubstituted ar(lower)alkoxy carbonyl [e.g. benzyloxycarbonyl, phenethylloxycarbonyl, benzhydryloxycarbonyl, 4-nitrobenzyloxycarbonyl, etc.], and the like, in which preferable one is unsubstituted lower alkoxy carbonyl and more preferable one is methoxycarbonyl or tert-butoxycarbonyl.

The carbamoyl substituted with lower alkyl may be methylcarbamoyl, ethylcarbamoyl, propylcarbamoyl, dimethylcarbamoyl, diethylcarbamoyl, N-methyl-N-ethylcarbamoyl and the like.

The carbamoyl substituted with aryl may be phenylcarbamoyl, naphthylcarbamoyl, lower alkyl-substituted phenylcarbamoyl [e.g. tolylcarbamoyl, xylylcarbamoyl, etc.] and the like.

The carbamoyl substituted with ar(lower)alkyl may be benzylcarbamoyl, phenethylcarbamoyl, phenylpropylcarbamoyl and the like, in which preferable one is benzylcarbamoyl.

The carbamoyl substituted with arylsulfonyl may be phenylsulfonylcarbamoyl, tolylsulfonylcarbamoyl and the like.

The carbamoyl substituted with lower alkylsulfonyl may be methylsulfonylcarbamoyl, ethylsulfonylcarbamoyl and the like.

The carbamoyl substituted with a heterocyclic group may be one substituted with a heterocyclic group as mentioned above for the definitions of general formulas [II-1] and [II-2].

The lower alkanoyl may be formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, hexanoyl and the like, in which preferable one is acetyl or pivaloyl.

The substituted or unsubstituted aroyl may be benzoyl, naphthoyl, toluoyl, di(tert-butyl)benzoyl, halo(lower)alkoxybenzoyl [e.g. trifluoromethoxybenzoyl, etc.] and the like, in which preferable one is benzoyl or trifluoromethoxybenzoyl.

The substituted or unsubstituted arylsulfonyl may be phenylsulfonyl, tolylsulfonyl, halophenylsulfonyl [e.g. fluorophenylsulfonyl, etc.] and the like, in which preferable one is fluorophenylsulfonyl.

The lower alkylsulfonyl may be methylsulfonyl, ethylsulfonyl and the like, in which preferable one is methylsulfonyl.

The cyclo(lower)alkylcarbonyl may be cyclo(C<sub>3</sub>-C<sub>6</sub>)alkylcarbonyl such as cyclopropylcarbonyl, cyclobutylcarbonyl, cyclopentylcarbonyl or cyclohexylcarbonyl, in which preferable one is cyclopropylcarbonyl.

The heterocyclic moiety in the term "a heterocycliccarbonyl" may be one mentioned above as a heterocyclic group for the definitions of general formulas [II-1] and [II-2].

Suitable "N-protective group" may be common N-protective group such as substituted or unsubstituted lower alkanoyl [e.g. formyl, acetyl, propionyl, trifluoroacetyl, etc.], lower alkoxycarbonyl [e.g. tert-butoxycarbonyl, tert-amylloxycarbonyl, etc.], substituted or unsubstituted aralkyloxycarbonyl [e.g. benzyloxycarbonyl, p-nitrobenzyloxycarbonyl, etc.], 9-fluorenylmethoxycarbonyl, substituted or unsubstituted arenesulfonyl [e.g. benzenesulfonyl, tosyl, etc.], nitrophenylsulfonyl, aralkyl [e.g. trityl, benzyl, etc.] or the like, in which preferable one is lower alkoxycarbonyl and more preferable one is tert-butoxycarbonyl.

Suitable "cyclic hydrocarbon" may be a saturated or unsaturated cyclic hydrocarbon such as cyclopentane, cyclohexane, benzene, naphthalene, indan, indene or the like.

Suitable "substituted-lower alkyl" may be lower alkyl

substituted with halogen, aryl, acyl, lower alkoxy, aryloxy and the like, in which preferable one is benzyl.

Suitable "heterocyclic ring" may be one which is a heterocyclic group, as mentioned above for the definitions of general formulas [II-1] and [II-2], added by hydrogen.

Suitable lower alkylene condensed with a cyclic hydrocarbon may be lower alkylene condensed with benzene and the like, in which preferable one is ethylene condensed with benzene.

Suitable lower alkylene condensed with a heterocyclic ring may be lower alkylene condensed with pyridine and the like, in which preferable one is ethylene condensed with pyridine.

Preferred "acyl" for  $R^4$  may be lower alkanoyl; lower alkoxy carbonyl; aroyl optionally substituted with halo(lower)alkoxy; arylsulfonyl optionally substituted with halogen; lower alkylsulfonyl; or cyclo(lower)alkylcarbonyl, in which more preferable one is acetyl, pivaloyl, methoxycarbonyl, tert-butoxycarbonyl, benzoyl, trifluoromethoxybenzoyl, fluorophenylsulfonyl, methylsulfonyl or cyclopropylcarbonyl.

Preferred "suitable substituent" as the substituent of lower alkyl, lower alkoxy, lower alkylamino, lower alkenyl, lower alkenyloxy, lower alkenylamino, lower alkynyl, lower alkynyloxy, lower alkynylamino, cyclo(lower)alkyl, cyclo(lower)alkyloxy, cyclo(lower)alkylamino, aryl, aryloxy, arylamino, a heterocyclic group or amino substituted with a heterocyclic group for  $R^7$  may be halo(lower)alkyl, halo(lower)alkoxy, lower alkenyl, lower alkynyl, lower alkylamino, acylamino, acyl, lower alkylsilyl, lower alkoxy, aryl, lower alkylendioxy, acyloxy, hydroxy, nitro, amino, cyano, halogen, aryloxy, lower alkylthio and the like.

Preferred "aryl which may be substituted with suitable substituent(s)" for  $R^7$  may be aryl optionally substituted with halogen, in which more preferable one is fluorophenyl.

Preferred "aryl amino which may be substituted with suitable substituent(s)" for  $R^7$  may be aryl amino optionally substituted with halogen, in which preferable one is phenyl amino or fluorophenyl amino.

Preferred "aryloxy which may be substituted with suitable substituent(s)" for  $R^7$  may be aryloxy optionally substituted with halogen, in which preferable one is fluorophenoxy.

Preferred "lower alkylene" for J may be methylene.

Preferred "lower alkyl" for R<sup>8</sup> in J may be methyl.

Preferred "N-protective group" for R<sup>8</sup> in J may be tert-butoxycarbonyl.

Preferred "suitable substituent" as the substituent of lower alkylene for E may be oxo, lower alkyl, hydroxy(lower)alkyl or acyl, in which more preferable one is oxo, dioxo, methyl, dimethyl, hydroxymethyl, or benzylcarbamoyl.

Preferred "lower alkylene" for E may be methylene, ethylene or trimethylene, and more preferable one is ethylene.

Preferred "lower alkyl" for R<sup>5</sup> and R<sup>6</sup> may be methyl.

Preferred "lower alkylene which R<sup>5</sup> and R<sup>6</sup> are taken together to form" may be ethylene or trimethylene.

Preferred "a cyclic hydrocarbon with which lower alkylene is condensed" may be benzene.

Another more preferred compound [II-2] is one having lower alkanoyl, lower alkoxycarbonyl, aroyl, aroyl substituted with halo(lower)alkoxy, lower alkylsulfonyl, arylsulfonyl, arylsulfonyl substituted with halogen, or cyclo(lower)alkylcarbonyl for R<sup>4</sup>, aryl or arylamino, each aryl of which may be substituted with halogen for R<sup>7</sup>, N for X, a single bond for J, and -CO- for Q.

Another most preferred compound [II-2] is one selected from the group consisting of

1-acetyl-4-(4-fluorophenylcarbamoyl)piperazine,

1-tert-butoxycarbonyl-4-(4-fluorophenylcarbamoyl)piperazine,

1-(4-fluorophenylcarbamoyl)-4-(4-trifluoromethoxybenzoyl)-piperazine and

1-methoxycarbonyl-4-(4-fluorophenylcarbamoyl)piperazine.

Suitable pharmaceutically acceptable salts of the compounds of general formulas [I], [II-1] and [II-2] are conventional non-toxic salts and include acid addition salt such as an inorganic acid addition salt [e.g. hydrochloride, hydrobromide, sulfate, phosphate, etc.], an organic acid addition salt [e.g. formate, acetate, trifluoroacetate, maleate, tartrate, methanesulfonate, benzenesulfonate, toluenesulfonate, etc.], a salt with an amino acid [e.g. aspartic acid salt, glutamic acid salt, etc.], a metal salt such as an alkali metal salt [e.g. sodium salt, potassium salt, etc.] and alkaline earth metal salt [e.g. calcium salt, magnesium salt, etc.] and the like.

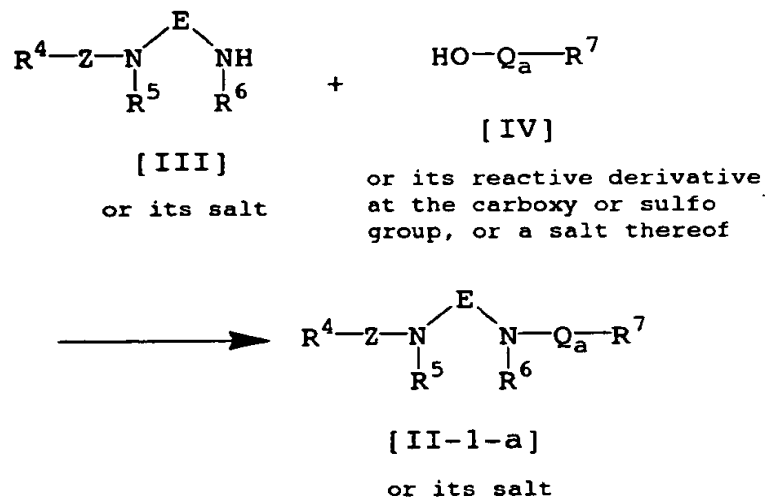
Compounds of the formula [I] and salts thereof can be prepared

according to the method disclosed in EP Publication No. 436734.

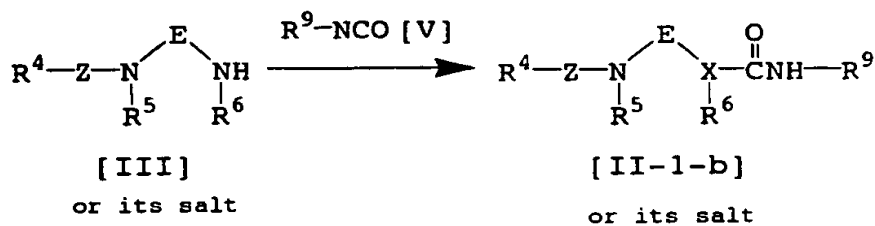
Compounds of the formula [II-1] including compounds [II-2] and salts thereof can be prepared by the processes as illustrated in the following reaction schemes.

5

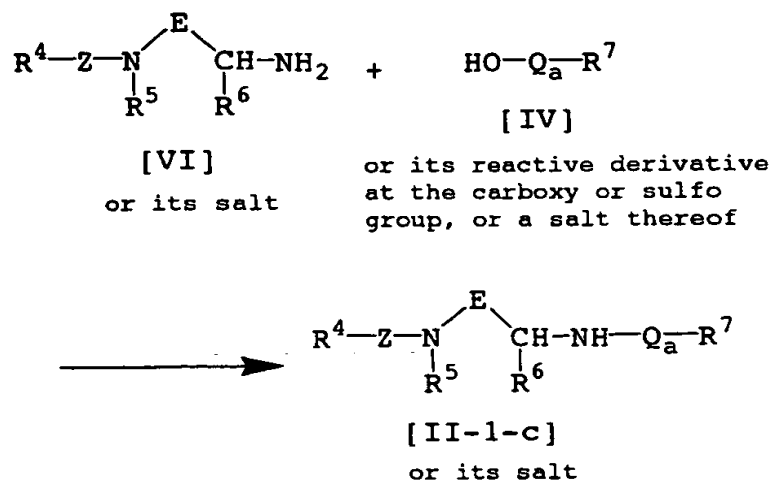
### Process 1



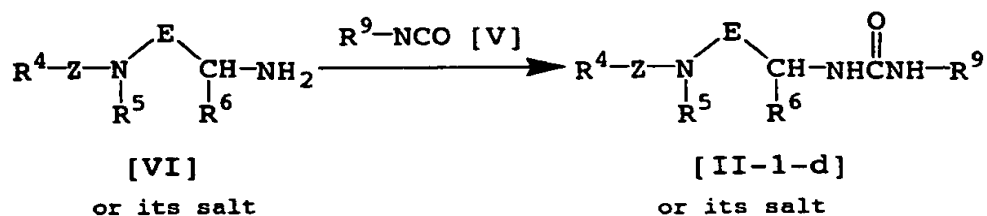
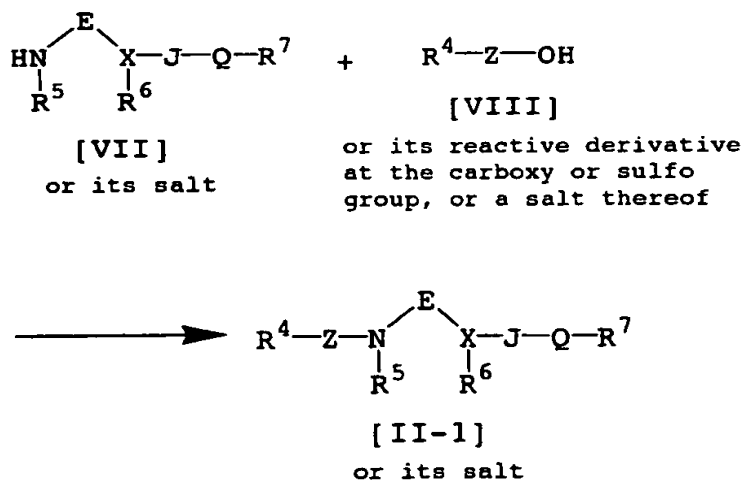
### 10 Process 2

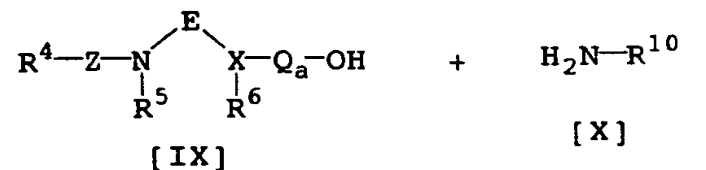


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Process 3Process 4

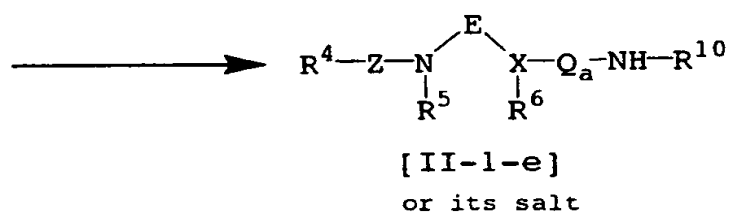
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Process 5

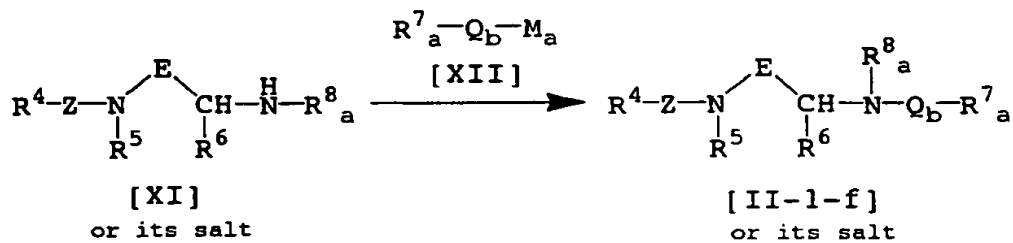
Process 6

or its reactive derivative  
at the carboxy or sulfo  
group, or a salt thereof

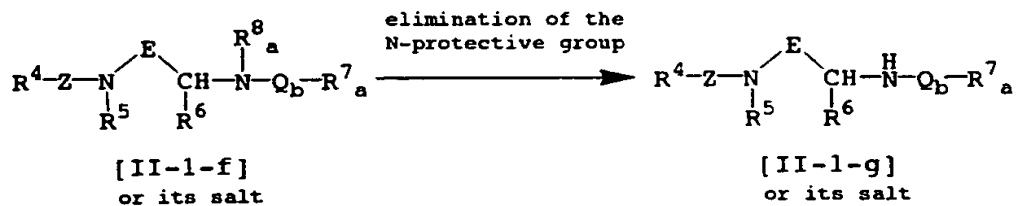
or its salt



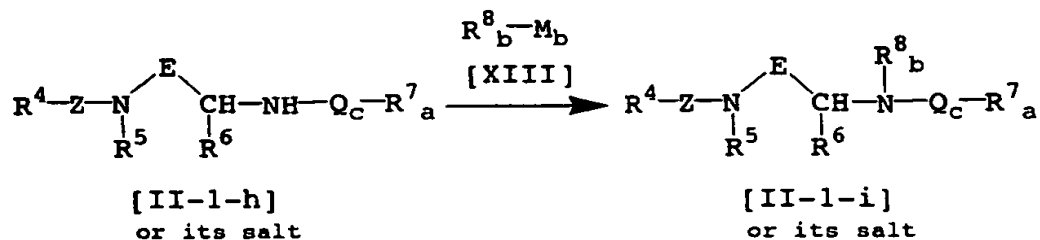
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Process 7Process 8

10

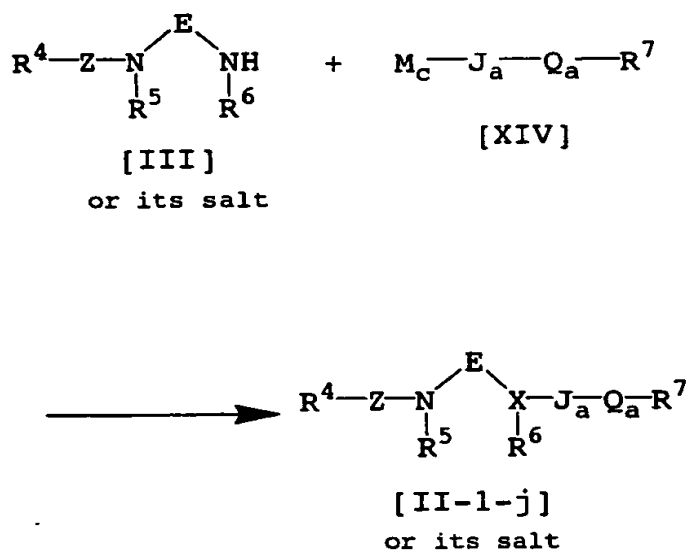


## Process 9



### Process 10

5



- 10 wherein R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, Z, E, Q, X and J are each as defined above,  
Q<sub>a</sub> is -CO- or -SO<sub>2</sub>-,  
R<sup>9</sup> is aryl which may be substituted with suitable  
substituent(s), or pyridyl,  
R<sup>10</sup> is lower alkyl, lower alkenyl, lower alkynyl,  
15 cyclo(lower)alkyl, aryl or a heterocyclic group, each of  
which may be substituted with suitable substituent(s),  
R<sup>8</sup><sub>a</sub> is an N-protective group,  
R<sup>7</sup><sub>a</sub> is lower alkyl, lower alkenyl, lower alkynyl,  
cyclo(lower)alkyl, aryl or a heterocyclic group, each of  
20 which may be substituted with suitable substituent(s),  
Q<sub>b</sub> is -CH<sub>2</sub>-, -CO-, or -SO<sub>2</sub>-,



- $M_a$  is an acid residue,  
 $Q_c$  is  $-CO-$ ,  
 $R^o_b$  is lower alkyl,  
 $M_b$  is an acid residue,  
 5  $M_c$  is an acid residue, and  
 $J_a$  is lower alkylene.

Suitable "acid residue" may be halogen [e.g. fluoro, chloro, bromo, iodo], arenesulfonyloxy [e.g. benzenesulfonyloxy, tosyloxy, etc.], alkanesulfonyloxy [e.g. mesyloxy, ethansulfonyloxy, etc.], and the like, in which preferable one is halogen.

The processes for preparing the compounds [II-1] including compounds [II-2] are explained in detail in the following.

#### Process 1

The compound [II-1-a] or its salt can be prepared by reacting a compound [III] or its salt with a compound [IV] or its reactive derivative at the carboxy or sulfo group, or a salt thereof.

Suitable salts of the compounds [II-1-a] and [III] may be the same as those exemplified for the compound [II-1].

Suitable salts of the compound [IV] and its reactive derivative at the carboxy or sulfo group may be metal salt or alkaline earth metal salt as exemplified for the compound [II-1].

Suitable reactive derivative at the carboxy or sulfo group of the compound [IV] may include an ester, an acid halide, an acid anhydride and the like. The suitable examples of the reactive derivatives may be an acid halide [e.g. acid chloride, acid bromide, etc.]; a symmetrical acid anhydride; a mixed acid anhydride with an acid such as aliphatic carboxylic acid [e.g. acetic acid, pivalic acid, etc.], substituted phosphoric acid [e.g. dialkylphosphoric acid, diphenylphosphoric acid, etc.]; an ester such as substituted or unsubstituted lower alkyl ester [e.g. methyl ester, ethyl ester, propyl ester, hexyl ester, trichloromethyl ester, etc.], substituted or unsubstituted ar(lower)alkyl ester [e.g. benzyl ester, benzhydryl ester, p-chlorobenzyl ester, etc.], substituted or unsubstituted aryl ester [e.g. phenyl ester, tolyl ester, 4-nitrophenyl ester, 2,4-dinitrophenyl ester, pentachlorophenyl ester, naphthyl ester, etc.], or an ester with N,N-dimethylhydroxylamine, N-hydroxysuccinimide, N-hydroxyphthalimide, 1-hydroxybenzotriazole, 1-hydroxy-6-chloro-1H-benzotriazole, or the like. These reactive

derivatives can be optionally selected according to the kind of the compound [IV] to be used.

The reaction is usually carried out in a conventional solvent such as water, acetone, dioxane, chloroform, methylene chloride, ethylene dichloride, tetrahydrofuran, acetonitrile, ethyl acetate, N,N-dimethylformamide, pyridine or any other organic solvent which does not adversely influence the reaction. Among these solvents, hydrophilic solvent may be used in a mixture with water.

The reaction is also preferably carried out in the presence of a conventional base such as triethylamine, diisopropylethylamine, pyridine, N,N-dimethylaminopyridine, etc., or a mixture thereof.

When the compound [IV] is used in a free acid form or its salt form in the reaction, the reaction is preferably carried out in the presence of a conventional condensing agent such as N,N'-dicyclohexylcarbodiimide, N-cyclohexyl-N'-morpholinoethylcarbodiimide, N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide, thionyl chloride, oxalyl chloride, lower alkoxy carbonyl halide [e.g. ethyl chloroformate, isobutyl chloroformate, etc.], 1-(p-chlorobenzenesulfonyloxy)-6-chloro-1H-benzotriazole, or the like.

The reaction temperature is not critical, and the reaction can be carried out under cooling to heating.

#### Process 2

The compound [II-1-b] or its salt can be prepared by reacting a compound [III] or its salt with a compound [V].

Suitable salts of the compound [II-1-b] and [III] may be the same as those exemplified for the compound [II-1].

This reaction is usually carried out in a solvent such as dioxane, tetrahydrofuran, benzene, toluene, chloroform, methylene chloride or any other organic solvent which does not adversely influence the reaction.

The reaction temperature is not critical, and the reaction is usually carried out under cooling to warming.

#### Process 3

The compound [II-1-c] or its salt can be prepared by reacting a compound [VI] or its salt with a compound [IV] or its reactive derivative at the carboxy or sulfo group, or a salt thereof.

Suitable salts of the compounds [II-1-c] and [VI] may be the same as those exemplified for the compound [II-1].

Suitable salts of the compound [VI] and its reactive derivative at the carboxy or sulfo group may be metal salt or alkaline earth metal  
5 salt as exemplified for the compound [II-1].

This reaction can be carried out in substantially the same manner as Process 1, and therefore the reaction mode and reaction condition [e.g. solvent, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 1.

10 Process 4

The compound [II-1-d] or its salt can be prepared by reacting a compound [VI] or its salt with a compound [V].

Suitable salts of the compound [II-1-d] and [VI] may be the same as those exemplified for the compound [II-1].

15 This reaction can be carried out in substantially the same manner as Process 2, and therefore the reaction mode and reaction condition [e.g. solvent, reaction temperature, etc.] of this reaction are to be referred to those explained in Process 2.

Process 5

20 The compound [II-1] or its salt can be prepared by reacting a compound [VII] or its salt with a compound [VIII] or its reactive derivative at the carboxy or sulfo group, or a salt thereof.

Suitable salt of the compound [VII] may be acid addition salt as exemplified for the compound [II-1].

25 Suitable salts of the compound [VIII] and its reactive derivative at the carboxy or sulfo group may be metal salt or alkaline earth metal salt as exemplified for the compound [II-1].

This reaction can be carried out in substantially the same manner as Process 1, and therefore the reaction mode and reaction condition  
30 [e.g. solvent, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 1.

Process 6

The compound [II-1-e] or its salt can be prepared by reacting a compound [IX] or its reactive derivative at the carboxy group or  
35 sulfo group, or a salt thereof with a compound [X] or its salt.

Suitable salts of the compounds [II-1-e], [IX] and its reactive derivative at the carboxy or sulfo group may be the same as those exemplified for the compound [II-1].

Suitable salt of the compound [X] may be acid addition salt as exemplified for the compound [II-1].

This reaction can be carried out in substantially the same manner as Process 1, and therefore the reaction mode and reaction condition [e.g. solvent, reaction temperature, etc.] of this reaction are to be referred to those as explained in Process 1.

#### Process 7

The compound [II-1-f] can be prepared by reacting a compound [XI] or its salt with a compound [XII].

Suitable salts of the compounds [II-1-f] and [XI] may be the same as those exemplified for the compound [II-1].

The present reaction is preferably carried out in the presence of base such as an alkali metal [e.g. lithium, sodium, potassium, etc.], alkaline earth metal [e.g. calcium, etc.], alkali metal hydride [e.g. sodium hydride, etc.], alkaline earth metal hydride [e.g. calcium hydride, etc.], the hydroxide or carbonate or bicarbonate of an alkali metal or an alkaline earth metal [e.g. potassium bicarbonate, etc.] and the like.

This reaction is usually carried out in a solvent such as N,N-dimethylformamide, diethyl ether, tetrahydrofuran, dioxane, benzene, toluene, acetonitrile or any other solvent which does not adversely influence the reaction.

The reaction temperature is not critical, and the reaction is usually carried out under cooling to heating.

#### Process 8

The object compound [II-1-g] or its salt can be prepared by subjecting a compound [II-1-f] or its salt to elimination reaction of the N-protective group.

Suitable salts of the compounds [II-1-f] and [II-1-g] may be acid addition salts as exemplified for the compound [II-1].

This reaction is carried out in accordance with a conventional method such as hydrolysis, reduction or the like.

The hydrolysis is preferably carried out in the presence of a base or an acid including Lewis acid.

Suitable base may include an inorganic base and an organic base such as an alkali metal [e.g. sodium, potassium, etc.], an alkaline earth metal [e.g. magnesium, calcium, etc.], the hydroxide or carbonate or bicarbonate thereof, hydrazine, alkylamine [e.g. methylamine,

trimethylamine, triethylamine, etc.], picoline, 1,5-diazabicyclo[4.3.0]non-5-ene, 1,4-diazabicyclo[2.2.2]octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, or the like.

Suitable acid may include an organic acid [e.g. formic acid, 5 acetic acid, propionic acid, trichloroacetic acid, trifluoroacetic acid, etc.], an inorganic acid [e.g. hydrochloric acid, hydrobromic acid, sulfuric acid, hydrogen chloride, hydrogen bromide, hydrogen fluoride, etc.] and an acid addition salt compound [e.g. pyridine hydrochloride, etc.].

10 The elimination using in trihaloacetic acid [e.g. trichloroacetic acid, trifluoroacetic acid, etc.] or the like is preferably carried out in the presence of cation trapping agents [e.g. anisole, phenol, etc.].

The reaction is usually carried out in a solvent such as water, 15 an alcohol [e.g. methanol, ethanol, etc.], methylene chloride, chloroform, tetrachloromethane, dioxane, tetrahydrofuran, a mixture thereof or any other solvent which does not adversely influence the reaction. A liquid base or acid can be also used as the solvent. The reaction temperature is not critical and the reaction is usually carried 20 out under cooling to heating.

The reduction method applicable for the elimination reaction may include chemical reduction and catalytic reduction.

Suitable reducing agents to be used in chemical reduction are a combination of metal [e.g. tin, zinc, iron, etc.] or metallic compound 25 [e.g. chromium chloride, chromium acetate, etc.] and an organic or inorganic acid [e.g. formic acid, acetic acid, propionic acid, trifluoroacetic acid, p-toluenesulfonic acid, hydrochloric acid, hydrobromic acid, etc.].

Suitable catalysts to be used in catalytic reduction are 30 conventional ones such as platinum catalysts [e.g. platinum plate, spongy platinum, platinum black, colloidal platinum, platinum oxide, platinum wire, etc.], palladium catalysts [e.g. spongy palladium, palladium black, palladium oxide, palladium on carbon, colloidal palladium, palladium on barium sulfate, palladium on barium carbonate, 35 etc.], nickel catalysts [e.g. reduced nickel, nickel oxide, Raney nickel, etc.], cobalt catalysts [e.g. reduced cobalt, Raney cobalt, etc.], iron catalysts [e.g. reduced iron, Raney iron, etc.], copper catalysts [e.g. reduced copper, Raney copper, Ullmann copper, etc.] and the like.

In case that the N-protective group is benzyl, the reduction is preferably carried out in the presence of a combination of palladium catalysts [e.g. palladium black, palladium on carbon, etc.] and formic acid or its salt [e.g. ammonium formate, etc.].

- 5       The reduction is usually carried out in a conventional solvent which does not adversely influence the reaction such as water, methanol, ethanol, propanol, N,N-dimethylformamide, or a mixture thereof. Additionally, in case that the above-mentioned acids to be used in chemical reduction are in liquid, they can also be used as a solvent.
- 10      Further, a suitable solvent to be used in catalytic reduction may be the above-mentioned solvent, and other conventional solvent such as diethyl ether, dioxane, tetrahydrofuran, etc. or a mixture thereof.

The reaction temperature of this reduction is not critical and the reaction is usually carried out under cooling the heating.

15      Process 9

The compound [II-1-i] or its salt can be prepared by reacting a compound [II-1-h] or its salt with a compound [XIII].

Suitable salts of the compounds [II-1-h] and [II-1-i] may be the same as those exemplified for the compound [II-1].

- 20       This reaction can be carried out in substantially the same manner as Process 7, and therefore the reaction mode and reaction condition [e.g. solvent, reaction temperature, etc.] of this reaction are to be referred to those explained in Process 7.

Process 10

- 25       The compound [II-1-j] or its salt can be prepared by reacting a compound [III] or its salt with a compound [XIV].

Suitable salts of the compounds [II-1-j] and [III] may be the same as those exemplified for the compound [II-1].

- 30       This reaction can be carried out in substantially the same manner as Process 7, and therefore the reaction mode and reaction condition [e.g. solvent, reaction temperature, etc.] of this reaction are to be referred to those explained in Process 7.

- 35       The compounds obtained by the above processes can be isolated and purified by a conventional method such as pulverization, recrystallization, column chromatography, reprecipitation, or the like.

It is to be noted that the compounds [I], [II-1], [II-2] and the other compounds may include one or more stereoisomer(s) such as

optical isomer(s) or geometrical isomer(s) due to asymmetric carbon atom(s) and double bond(s), and all of such isomers and mixture thereof are included within the scope of this invention.

5 Additionally, it is to be noted that any solvate [e.g. enclosure compound (e.g. hydrate, etc.)] of the compound [I], [II-1], [II-2] and pharmaceutically acceptable salts thereof is also included within the scope of this invention.

10 With regard to the brain somatostatin release promoting property, the brain somatostatin release promoting property is said to be expressed and the compound is said to have a brain somatostatin activation property, when the release amount of somatostatin by depolarization stimulation is increased due to a pretreatment of a hippocampal slice with a certain compound, as compared to when the pretreatment with the compound is  
15 void.

With regard to the property of increasing biosynthesis of somatostatin, the property of increasing biosynthesis of somatostatin in nerve cells is said to be expressed and the compound is said to have a brain somatostatin activation property, when a somatostatin  
20 content of the nerve cells is increased in the nerve cells due to a pretreatment with a certain compound, as compared to when the pretreatment with the compound is void.

With regard to the somatostatin receptor activation property, the somatostatin receptor activation property is said to be expressed  
25 and the compound is said to have a brain somatostatin activation property, when binding of somatostatin labeled with a radioisotope (e.g., <sup>125</sup>I and the like) to a somatostatin receptor in the nerve cells decreases due to a pretreatment with a certain compound, as compared to when the pretreatment with the compound is void.

30 With regard to the potentiation property of the expression of somatostatin property, the potentiation property of the expression of somatostatin property is said to be expressed and the compound is said to have a brain somatostatin activation property, when the activity of somatostatin degrading enzyme in the nerve cells is suppressed due  
35 to a pretreatment with a certain compound, as compared to when the pretreatment with the compound is void.

With regard to the potentiation property of the somatostatin signal transmission, the potentiation property of the somatostatin

signal transmission is said to be expressed and the compound is said to have a brain somatostatin activation property, when the amount of intracellular phosphorylated protein in the nerve cells changes due to a pretreatment with a certain compound, as compared to when the  
5 pretreatment with the compound is void.

With regard to the property of suppressing the negative feedback mechanism of brain somatostatin release, the property of suppressing the negative feedback mechanism of brain somatostatin release is said to be expressed and the compound is said to have a brain somatostatin  
10 release promoting property, when the suppressive action on the membrane potential dependent calcium current by somatostatin in the hippocampus pyramidal cells is released due to a pretreatment with a certain compound.  
**Agent for expression of long-term potentiation of synaptic transmission**

The compound having a brain somatostatin activation property  
15 expresses the long-term potentiation of synaptic transmission.

Therefore, this compound is used as an agent for expression of long-term potentiation of synaptic transmission for mammals such as human, dog, cow, horse, rat, guinea pig and the like.

In the present invention, an agent for expression of long-term  
20 potentiation of synaptic transmission means a compound capable of inducing potentiation of general neurotransmission efficiency after high frequency and short time stimulation and sustaining the increased transmission efficiency for a long time.

The site where the long-term potentiation of synaptic  
25 transmission is expressed is subject to no limitation as long as it is a nerve system present in the hippocampus. Examples thereof include nerve systems present in brain, such as cerebral cortex, corpus amygdaloideum and the like, with preference given to the mossy fiber - CA3 pathway, perforant path-dentate gyrus pathway and Schaffer  
30 collaterals-CA1 pathway.

The agent for expression of long-term potentiation of synaptic transmission of the present invention is effective for the prophylaxis and/or treatment of cerebral diseases such as dementia (e.g., senile dementia, Alzheimer's dementia, dementia associated with various  
35 diseases such as cerebral vascular dementia, cerebral post-traumatic dementia, dementia due to brain tumor, dementia due to chronic subdural hematoma, dementia due to normal pressure hydrocephalus, post-meningitis dementia, Parkinson's disease type dementia, and the



like), amnesia, manic-depressive psychosis, schizophrenia, Parkinson's disease, psychosomatic disease, and the like, particularly for the prophylaxis and/or treatment of dementia and amnesia.

The long-term potentiation of synaptic transmission is evaluated according to the following criteria.

In a long-term potentiation phenomenon observed in the hippocampus of an animal, namely, guinea pig, which is observed according to the method conventionally known (Matsuoka et al., *Brain Research*, vol. 553, p. 188, 1991), when a pretreatment of hippocampus with a certain compound (bringing hippocampus into contact with the compound) leads to the augmentation of LTP, after tetanic stimulation, of potential generated from the CA3 field pyramidal cell caused by stimulation of mossy fiber to not less than 120%, preferably not less than 140%, more preferably not less than 160%, still more preferably not less than 180%, most preferably not less than 200%, when that without the pretreatment with the compound is 100%, the long-term potentiation of synaptic transmission is said to be expressed, and this compound can be said to be an agent for expression of long-term potentiation of synaptic transmission.

Alternatively, in a long-term potentiating phenomenon observed in the hippocampus of an animal, namely, guinea pig, which is observed according to the method conventionally known (Matsuoka et al., *Brain Research*, vol. 553, p. 188, 1991), when a long-term potentiation phenomenon is expressed in a sustained manner for not less than 10 minutes, preferably not less than 20 minutes, more preferably not less than 30 minutes, most preferably not less than 60 minutes due to a pretreatment with a certain compound, a long-term potentiation of synaptic transmission is said to be expressed and this compound can be said to be an agent for expression of long-term potentiation of synaptic transmission.

Note that variations in numerical values to the degree that those of ordinary skill in the art consider substantially the same statistically as the above-mentioned numerical values should be construed as corresponding to the above-mentioned numerical values.

As used herein, the pretreatment with a certain compound can be conducted by, for example, immersing the compound generally for 25 minutes in an outer solution containing hippocampus therein, and removing the compound by a method such as washing.

The test method for the evaluation of the above-mentioned long-term potentiation of synaptic transmission may be a method conventionally known or a method analogous thereto, with preference given to the following method.

5       Round slices are prepared from the hippocampus removed from an animal, for example, guinea pig. The slices are 500  $\mu$ m in thickness. Each slice is placed in a container such as a perfusion chamber and the population spikes are extracellularly recorded in the following manner while perfusing with an artificial cerebrospinal fluid at 33  
10   - 34°C at a flow rate of 1.8 - 2.0 ml/min.

At this time, the test compound is added to the perfusate so that the concentration of the test compound in each container would be a serially diluted concentration, and the test compound is applied to the hippocampal slices from 21 minutes before the tetanic stimulation  
15   to 4 minutes thereafter.

The artificial cerebrospinal fluid to be used here is exemplified by those used in the screening method in the present invention to be mentioned later.

Mossy fiber is stimulated with a stimulation electrode at a voltage  
20   of not more than 10 V, frequency of 0.2 Hz, and the population spikes in the CA3 field pyramidal cell layer are recorded every 5 minutes. When the population spikes to be recorded are stabilized, tetanic stimulation is applied for the induction of LTP. The tetanic stimulation includes stimulation at the same voltage and a frequency of 33 Hz for  
25   5 seconds. The height of from the negative peak to the next positive peak of the obtained population spikes after stimulation is measured and taken as an amplitude of population spikes. The degree of LTP can be expressed by a potential variation (%) showing an increase in the amplitude of population spikes after tetanic stimulation relative to  
30   the average of 4 measurements of the amplitudes of population spikes before tetanic stimulation. As the index of the property of a drug, the area in the graph of time lapse versus potential variation, between the graph from 12 minutes to 62 minutes after tetanic stimulation and the line where the potential variation is 0%, may be calculated and  
35   taken as the magnitude of LTP.

Each value of the potential variation and the magnitude of LTP which is calculated based on the potential variation as mentioned above is subjected to one-way analysis of variance and Dunnett's multiple

comparison test, and compared based on the above-mentioned evaluation criteria, whereby the long-term potentiation of synaptic transmission can be evaluated.

#### screening method

5       The screening method of the present invention comprises screening of the test compound to be screened (hereinafter to be abbreviated as a test compound) using the somatostatin releasing property as an index.

10       The screening method of the agent for expression of long-term potentiation of synaptic transmission of the present invention, particularly, an anti-dementia agent and an anti-amnesia agent, is a selection method of the test compound characteristically comprising bringing human or animal nerve cells into contact with a test compound, measuring an amount of somatostatin released from the nerve cells and  
15       a release time thereof, measuring an amount of somatostatin released from the nerve cells and a release time thereof in the absence of a contact with the test compound, and comparing the amounts and the times to measure the amount of somatostatin released from the nerve cells and the release time thereof caused by the contact with the test compound.

20       The above-mentioned nerve cells include, for example, hippocampus, preferably hippocampal slices, primary culture nerve cell, nerve cell strain, oocyte and the like.

      The contact with the test compound can be conducted by, for example, immersing the test compound for 20 - 120 minutes in an outer solution  
25       in which the nerve cells are immersed and removing the test compound by washing and the like.

      Preferred screening method is as follows.

      Hippocampal slices are prepared from an animal (e.g., rat, guinea pig, mouse and the like) by a method conventionally known or a method  
30       analogous thereto. The slices are generally 100 - 600  $\mu\text{m}$ , preferably 300 - 400  $\mu\text{m}$ , in thickness. While the direction of slicing is not particularly limited, they are preferably transverse slices or longitudinal slices. For example, the slices are placed in a container such as a perfusion chamber, by generally 30 slices, more preferably  
35       20 slices, and perfused with an artificial cerebrospinal fluid while incubating at generally 20 - 37°C, preferably 36 - 37°C. The perfusate is exchanged every 5 - 20 minutes, preferably 10 - 15 minutes. Fractions are obtained from each cycle, which step is repeated 10 to 20 times,

preferably 15 times. While repeatedly obtaining the fractions, the test compound is added to the perfusate to serially diluted concentrations that are within the range of preferably  $10^{-10}$  -  $10^{-5}$  M, more preferably  $10^{-9}$  -  $10^{-6}$  M, whereby fractions containing the test compound at respective concentrations are obtained. The test compound is immersed in perfusate for generally 20 - 120 minutes, preferably 80 minutes.

Generally 0 - 60 minutes, preferably 10 - 30 minutes, after the addition of the test compound, stimulation is preferably added. The stimulation is applied for generally 5 - 20 minutes, preferably 10 - 15 minutes. It is important that the stimulation be applied after the addition of the test compound, but the stimulation may be applied while the test compound is present in the perfusate or after the test compound is removed by washing with a perfusate.

The somatostatin in the perfusate of each fraction obtained as above is quantified according to a treatment method conventionally known (for example, perfusate is lyophilized and subjected to a radioimmunoassay). After the completion of the step for obtaining the fractions, the somatostatin remaining in hippocampal slices is extracted by a conventional method, which amount is similarly quantified.

The composition of the artificial cerebrospinal fluid to be used as the perfusate in the above-mentioned screening method can be modified as appropriate depending on the test conditions and test compound and the like to be used. Preferable composition includes the following. Artificial cerebrospinal fluid composition: NaCl, 124 mM; KCl, 5 mM;  $\text{KH}_2\text{PO}_4$ , 1.24 mM;  $\text{MgSO}_4$ , 1.3 mM;  $\text{CaCl}_2$ , 2.4 mM;  $\text{NaHCO}_3$ , 26 mM; D-glucose, 10 mM

This perfusate is used with saturation with a mixed gas of oxygen and carbon dioxide, such as a mixed gas of oxygen (95%) and carbon dioxide (5%), typically used for pharmacological experiments.

The above-mentioned screening method may be modified as necessary.

The amount of somatostatin quantified is compared with the amount when the contact with the test compound is void, based on the following criteria, whereby the somatostatin release promoting property of the test compound can be evaluated.

The screening method of the present invention is preferably

conducted under stimulation as mentioned above. Such stimulation is a model of a specific stimulation related to learning or tetanic stimulation. The stimulation is not particularly limited as long as the nerve cells present in the hippocampal slice are excited. Specific examples thereof include stimulation by potassium ion, electric stimulation, depolarization stimulation, stimulation with a drug and the like. When a mere addition of the test compound does not lead to the somatostatin release property and the test compound shows somatostatin release property only upon stimulation, it can be a confirmation that the nerve cells are free from influence of this test compound as long as no stimulation is involved, thus ensuring the safety of this compound.

The somatostatin release property is evaluated based on the following criteria.

In the screening method of the present invention, when the somatostatin release amount due to the contact with the test compound increases by not less than 10%, preferably not less than 20%, more preferably not less than 30%, still more preferably not less than 40%, most preferably not less than 50%, as compared to the release amount when the contact with the test compound is void (which is taken as 100%), the test compound is said to have a somatostatin release promoting property. It is preferable that the somatostatin release amount upon stimulation mentioned above be evaluated according to the above-mentioned criteria. It is appreciated that variations in numerical values to the degree that those of ordinary skill in the art consider substantially the same statistically as the above-mentioned numerical values should be construed as corresponding to the above-mentioned numerical values.

The test compound to be subject to the screening in the present invention is free of any particular limitation and may be selected from natural product, chemically synthesized compound, nucleic acid, peptide, antibody and the like obtained by genetic engineering and their libraries. The test compound is preferably a pure substance, but may be a mixture or racemic compound. The test compound may be also modified to label with radioisotope or may contain modification made during construction of library. The obtained test compound can be optimized by chemical synthetic method and the like.

By selecting the test compounds using the screening method of

the present invention, an agent for expression of long-term potentiation of synaptic transmission, particularly an anti-dementia agent, an anti-amnesia agent and the like can be screened.

5 The test compound selected by the screening method of the present invention and a compound obtained by optimizing this compound are all encompassed in the scope of the present invention.

10 The compound of the present invention having a brain somatostatin activation property can be used in the dosage form of a solid, semi-solid or liquid preparation in conjunction with organic or inorganic carrier or excipient, which is suitable for rectal administration, pulmonary (pernasal or buckle inhalation), nasal drop, eyedrop, external (local), oral or parenteral (subcutaneous, intravenous or intramuscular) administration and the like, direct administration to diseased region, such as brain, spinal fluid, cerebroventricle and the like, or  
15 inhalation.

A compound having a brain somatostatin activation property can be admixed with pharmaceutically acceptable substantially non-toxic carrier or excipient conventionally used for dosage forms suitable for use, such as tablets, pellets, troches, capsules, suppositories,  
20 cream, ointment, aerosol, inhalable powder medicine, liquid, emulsion, suspension, and the like. Where necessary, auxiliary, stabilizer, tackifier, coloring agent and flavor can be used.

The agent for the expression of long-term potentiation of synaptic transmission, particularly an anti-dementia agent and an anti-amnesia  
25 agent, of the present invention can be produced by a method conventionally used in the pertinent field. Where necessary, a method routinely used in this technical field can be used for the production of these drugs for an improved bioavailability.

30 The agent for the expression of long-term potentiation of synaptic transmission, particularly an anti-dementia agent and an anti-amnesia agent, of the present invention is preferably administered intravenously (inclusive of addition into infusion), intramuscularly or orally when applying to humans or animals.

35 The agent for the expression of long-term potentiation of synaptic transmission, particularly an anti-dementia agent and an anti-amnesia agent, of the present invention is contained in a preparation in an amount sufficient to provide a desired prophylactic and/or treatment effect on the progression and conditions of diseases.

The amount and administration route of the compound having a brain somatostatin activation property are subject to variation depending on the kind of compound, age and conditions of the patients to be the subject of the prophylaxis and/or treatment. When compound 1 is used, for example, the daily dose is 0.1 - 10 mg/kg body weight by oral administration, which is given once to several times a day for the treatment and/or prophylaxis of the aforementioned diseases.

The present invention is explained in more detail in the following by way of Examples that do not limit the present invention.

10

#### Examples

**Experimental Example 1:** Effect of compound 1 on somatostatin release from rat hippocampal slice

##### (1) method

Rat hippocampal slices (thickness 350  $\mu$ m, round slice) were prepared by a standard method. Twenty rat hippocampal slices were placed in a perfusion chamber, incubated at 37°C and perfused by a batch method while exchanging the incubation buffer every 10 minutes. The incubation buffer used had the composition as noted below. A mixed gas of oxygen (95%) and carbon dioxide (5%) was used to saturate the buffer.

20 Composition of incubation buffer: NaCl, 124 mM; KCl, 5 mM;  $\text{KH}_2\text{PO}_4$ , 1.24 mM;  $\text{MgSO}_4$ , 1.3 mM;  $\text{CaCl}_2$ , 2.4 mM;  $\text{NaHCO}_3$ , 26 mM; D-glucose, 10 mM

Perfusion for 150 minutes gave fractions 1-15. To fraction 9 was applied a high  $\text{K}^+$  (50 mM) stimulation. Compound 1 was added to fractions 7 - 15 to the concentration of  $10^{-9}$  M,  $10^{-8}$  M,  $10^{-7}$  M,  $10^{-6}$  M, respectively. Nothing was added to control group. The respective fractions thus obtained were concentrated by lyophilization and somatostatin in the perfusate was quantified by radioimmunoassay (RIA). After the completion of the experiment, somatostatin remaining in the slices was extracted by a conventional method and quantified by radioimmunoassay. The somatostatin amount released by high  $\text{K}^+$  (50 mM) stimulation was calculated and the amount of somatostatin released due to the property of compound 1 was measured.

Somatostatin release (%) by compound 1 at each concentration was calculated as in the following. The somatostatin amount of each fraction was expressed by the percentage (%) relative to the somatostatin residual amount at the time the fraction was obtained. The value of fraction 8 immediately before high  $\text{K}^+$  (50 mM) stimulation was taken as the base and the values exceeding the base value were added with

regard to fraction 9 and the subsequent peak fractions exceeding the base value to give somatostatin release (%). The number of the test samples measured was 10 or 11. Each value (%) was expressed by mean  $\pm$  S.E.M. The property of compound 1 was subjected to Dunnett's multiple comparison test relative to control group.

## (2) Result

The results are shown in Fig. 1. The compound 1 promoted somatostatin release when stimulated at high  $K^+$  (50 mM), and the property was significant at  $10^{-7}$  M and  $10^{-6}$  M. The concentration dependency was similar to the long-term potentiation in Experimental Example 2 to be mentioned later. By the use of the screening method of the present invention, compound 1 was shown to have a somatostatin release promoting property on hippocampus.

**Experimental Example 2: Effect of compound 1 on LTP in mossy fiber - CA3 field pyramidal cell of hippocampal slice**

### (1) method

Slices (thickness 500  $\mu$ m, round slice) were prepared from hippocampus removed from male Hartley guinea pigs (body weight 220 - 350 g) and population spikes were extracellularly recorded. The hippocampal slices were perfused with an artificial cerebrospinal fluid (33-34°C, composition: NaCl, 124 mM; KCl, 5 mM;  $KH_2PO_4$ , 1.24 mM;  $MgSO_4$ , 1.3 mM;  $CaCl_2$ , 2.4 mM;  $NaHCO_3$ , 26 mM; D-glucose, 10 mM) saturated with a mixed gas of oxygen (95%) and carbon dioxide (5%) at flow rate of 1.8 - 2.0 ml/min. Mossy fiber was stimulated with a stimulating electrode at a voltage of not more than 10 V, frequency of 0.2 Hz and the population spikes in the CA3 field pyramidal cell layer was recorded every 5 minutes. When the population spikes to be recorded were stabilized, tetanic stimulation for induction of LTP was applied. The tetanic stimulation includes stimulation at the same voltage of not more than 10 V, as when the stimulation was applied at a frequency of 0.2 Hz, but upon increase of the frequency to 33 Hz for 5 seconds. The height of from negative peak to the next positive peak of the obtained population spikes after stimulation was measured and taken as an amplitude (population spike amplitude; PSA). The degree of LTP was expressed by a potential variation (%) by the increase in the amplitude of population spikes after tetanic stimulation relative to the average of 4 measurements of the amplitudes of population spikes obtained before tetanic stimulation. Each value was expressed by mean  $\pm$  S.E.M (the



number of slices tested was 3 to 8). As the index of the property of a drug, the area (%·min) between the graph from 12 minutes to 62 minutes after tetanic stimulation and the line where the potential variation is 0%, in the time lapse graph of potential variation, was calculated and taken as the magnitude of LTP. Compound 1 was dissolved in and diluted with distilled water and added to perfusate to the concentration of  $10^{-9}$  M,  $10^{-8}$  M,  $10^{-7}$  M and  $10^{-6}$  M, respectively, and applied to a hippocampal slice for 25 minutes from 2 minutes before the tetanic stimulation to 4 minutes thereafter. Nothing was added to perfusate for control group.

The property of compound 1 relative to the magnitude of LTP was tested by one-way analysis of variance and Dunnett's multiple comparison test.

## (2) Results

From the potential variation with the lapse of time, compound 1 was found to have shown no significant influence of the response of base before tetanic stimulation. As is can be seen from Fig. 2 showing the magnitude of LTP, compound 1 increased potential variation after tetanic stimulation at  $10^{-7}$  M and  $10^{-6}$  M, wherein its action was maximal at  $10^{-7}$  M. From the foregoing, it was shown that compound 1 had a promoting property specific to the mechanism necessary for the occurrence of phenomenon of long-term potentiation of synaptic transmission after tetanic stimulation.

The hippocampal slice obtained from the guinea pig treated with cysteamine (200 mg/kg) did not show LTP enhancing property by compound 1 at  $10^{-7}$  M. This reveals that hippocampus endogeneous somatostatin activation property is involved in the LTP enhancing property by compound 1.

**Experimental Example 3: Effect of compound 1 on voltage-dependent  $\text{Ca}^{2+}$  current of rat hippocampus pyramidal cell**

### (1) method

Hippocampal slices were prepared from the brains of 5- to 14-day-old male Wistar rats and the nerve cells were quickly isolated by trypsin enzyme treatment method. According to the whole cell patch-clamp method, the membrane potential of hippocampus pyramidal cell was fixed at -80 mV and the membrane potential-dependent potassium current ( $I_{\text{K}}$ ) was measured in  $\text{Cs}^+$  electrode inner solution and  $\text{Ba}^{2+}$  bath outer solution. The resistance of the electrode was 2 - 4 MΩ.

Compound 1 was added to bath outer solution concentrations of 0.01  $\mu\text{M}$ , 0.1  $\mu\text{M}$  and 1.0  $\mu\text{M}$ , respectively, and perfused with the bath outer solution. Nothing was added to the bath outer solution for the control group. The maximal value of the current (peak amplitude) was measured, and calculated as the variation (%) relative to the value before addition of compound 1. Each value (%) was expressed by mean  $\pm$  S.E.M. The number of measurements was 7. The property of compound 1 was evaluated by Dunnett's multiple comparison test relative to the control group.

(2) Result

The results are shown in Fig. 3. Compound 1 significantly promoted membrane potential-dependent calcium current at concentrations of 0.01  $\mu\text{M}$ , 0.1  $\mu\text{M}$  and 1.0  $\mu\text{M}$  as compared to the control group, and showed bell-shape dose dependency. Therefore, compound 1 was shown to have a promoting action on the voltage-dependent calcium channel of hippocampus pyramidal cells.

**Experimental Example 4: Effect of somatostatin and compound 1 on voltage-dependent  $\text{Ca}^{2+}$  current of rat hippocampus pyramidal cell (1) method**

In the same manner as in Experimental Example 3 except that compound 1 and somatostatin were added in such a manner that the concentration of somatostatin in the bath outer solution was  $10^{-7}$  M when somatostatin alone was added, and the concentration of compound 1 in the bath outer solution was  $10^{-7}$  M and the concentration of somatostatin in the bath outer solution was  $10^{-7}$  M when both compound 1 and somatostatin were added, the experiment was conducted. Nothing was added to the control group. The maximal value of each current was measured, and calculated as the variation (%) to that prior to addition. Each value (%) was expressed by mean  $\pm$  S.E.M. The number of measurements of the group added with somatostatin was 11 and that of the group added with both compound 1 and somatostatin was 7. The both groups were subjected to Dunnett's multiple comparison test.

(2) Result

The results are shown in Fig. 4. While somatostatin evidently suppressed membrane potential-dependent calcium current, suppressive property of somatostatin was completely released in the presence of compound 1. Hence, compound 1 was shown to release the suppressive property of somatostatin on the voltage-dependent calcium channel of hippocampus pyramidal cell and has a promoting property. From the

results of Experimental Example 3 and this Experimental Example, compound 1 was shown to suppress the negative feedback mechanism of brain somatostatin release.

5       The compounds [II-1] including compounds [II-2] used in the present invention and preparation methods thereof are explained in detail by way of the following Reference preparations and Reference examples. It is needless to say that those Reference preparations and Reference examples do not limit the present invention.

10   Reference preparation 1

To a solution of 1-benzyl-4-aminopiperidine (50 g) in water (360 ml) was added a solution of di-tert-butyl dicarbonate (61 g) in acetone (360 ml) dropwise under cooling on an ice-water bath. After stirring for 2.5 hours, a precipitate was collected on a filter, washed with  
15   water, and dried. The crude product was poured into a mixture of diisopropyl ether (200 ml) and n-hexane (200 ml) and the mixture was stirred. After filtration, O-tert-butyl N-(1-benzylpiperidin-4-yl)-carbamate (66.9 g) was obtained.

20       NMR (DMSO- $d_6$ ,  $\delta$ ): 1.2-1.5 (2H, m), 1.37 (9H, s), 1.66 (2H, br d, J=9.9Hz), 1.91 (2H, br t J=10.7Hz), 2.73 (2H, distorted d, J=11.8Hz), 3.2 (1H, m), 3.41 (2H, s), 6.75 (1H, d, J=7.8Hz), 7.1-7.4 (5H, m)

MASS (APCI)(m/z): 291

Reference preparation 2

25       To a mixture of O-tert-butyl N-(1-benzylpiperidin-4-yl)-carbamate (45 g) and 10% palladium on carbon (50% wet, 9 g) in methanol (1 l) was bubbled hydrogen gas under stirring at ambient temperature. The catalyst was removed by glass filter and the solvent was removed under reduced pressure. After rinse with diisopropyl ether,  
30   O-tert-butyl N-(piperidin-4-yl)carbamate (28.35 g) was obtained. The washed solvent was removed under reduced pressure, and the residue was rinsed with diisopropyl ether. The second fraction of O-tert-butyl N-(piperidin-4-yl)carbamate (344 mg) was obtained.

35       NMR (DMSO- $d_6$ ,  $\delta$ ): 1.18 (2H, ddd, J=3.8, 11.8, 11.8Hz), 1.37 (9H, s), 1.62 (2H, distorted d, J=10.8Hz), 1.85 (1H, m), 2.38 (2H, dt, J=2.2, 12.0Hz), 2.86 (2H, distorted d, J=12.3Hz), 3.2 (1H, m), 6.72 (1H, br d)

MASS (APCI)(m/z): 201

Reference preparation 3

To a suspension of O-tert-butyl N-(piperidin-4-yl)carbamate (4.0 g) in dichloromethane (40 ml) were added pyridine (1.94 ml), dichloromethane (40 ml), acetic anhydride (20.8 ml) and then  
5 N,N-dimethylaminopyridine (0.1 g) at ambient temperature. After stirring for 3 hours, the mixture was washed with 0.1N hydrochloric acid, water, and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. After rinse with diisopropyl ether, O-tert-butyl N-(1-acetylpiperidin-4-yl)carbamate  
10 (4.01 g) was obtained.

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.23 (2H, m), 1.38 (9H, s), 1.70 (2H, distorted t, J=11.4Hz), 1.97 (3H, s), 2.64 (1H, br t, J=11.1Hz), 3.04 (1H, dt, J=2.8, 11.5Hz), 3.42 (1H, m), 3.72 (1H, br d, J=15.0Hz), 4.19 (1H, br d, J=13.1Hz), 6.86 (1H, d, J=7.5Hz)

15 MASS (APCI)(m/z): 243

Reference preparation 4

To a solution of O-tert-butyl N-(1-acetylpiperidin-4-yl)-carbamate (2.42 g) in dichloromethane (24 ml) was added 4N hydrogen chloride in dioxane (24 ml). The solvents were removed under reduced  
20 pressure. After rinse with diisopropyl ether, 1-acetyl-4-amino-piperidine hydrochloride (2.02 g) was obtained.

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.41 (2H, m), 1.93 (2H, distorted t), 2.00 (3H, s), 2.60 (1H, br t, J=10.4Hz), 3.06 (1H, br t, J=11.3Hz), 3.12 (1H, m), 3.84 (1H, br d, J=14.0Hz), 4.34 (1H, br d, J=13.0Hz),  
25 8.32 (3H, br s)

MASS (APCI)(m/z): 143

Reference preparation 5

To a solution of phenyl chloroformate (5.64 g) in dichloromethane (70ml) was added a solution of 4-aminopyridine (2.84 g) and triethylamine  
30 (5.02 ml) in dichloromethane (100 ml) dropwise under cooling on an ice-water bath. After stirring for 1 hour, the solvents were removed under reduced pressure. A residue was diluted with dichloromethane (200 ml) and water (200 ml). An organic phase was separated and washed with water and brine. After drying with magnesium sulfate, the solvents  
35 were removed under reduced pressure. The reaction mixture was diluted with diisopropyl ether and the precipitates were filtered. After rinse with diethyl ether, O-phenyl N-(4-pyridyl)carbamate (5.07 g) was obtained.

NMR (CDCl<sub>3</sub>,  $\delta$ ): 7.17 (2H, m), 7.27 (1H, m), 7.3-7.5 (4H, m), 8.50 (2H, dd, J=1.4, 5.0Hz), 8.06 (1H, s)

MASS (APCI)(m/z): 215

Reference preparation 6

5 A solution of sulfonyl chloride (3.55 ml) in chloroform (45 ml) was added a solution of 1-acetylpiperazine (5.66 mg) and triethylamine (6.16 ml) in chloroform (15 ml) dropwise under cooling on an ice-water bath. After stirring for 6 hours, a precipitate was collected by filtration. After drying over sodium hydroxide, 1-acetylpiperazine-4-sulfonyl chloride (2.43 g) was obtained.

NMR (CDCl<sub>3</sub>,  $\delta$ ): 2.15 (3H, s), 3.35 (4H, m), 3.69 (2H, t, J=5.1Hz), 3.83 (2H, br s)

MASS (APCI)(m/z): 227

Reference preparation 7

15 To a solution of 1-benzyl-4-aminopiperidine (1.13 g) in dichloromethane (10 ml) were added a solution of 4-fluorobenzoyl chloride (0.99 g) in dichloromethane (1 ml) and diisopropylethylamine (1.09 ml) under cooling on an ice-water bath. The mixture was warmed to ambient temperature slowly under stirring. The mixture was diluted  
20 with dichloromethane and washed with water, saturated aqueous sodium hydrogen carbonate, water, and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 100 ml, dichloromethane:methanol = 15:1). After rinse with diisopropyl ether  
25 - n-hexane (1:1), N-(1-benzylpiperidin-4-yl)-4-fluorobenzamide (1.31 g) was obtained.

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.4-1.7 (2H, m), 1.7-1.9 (2H, m), 2.01 (2H, br t, J=10.7Hz), 2.81 (2H, br d, J=11.6Hz), 3.46 (2H, s), 3.73 (1H, m), 7.2-7.4 (7H, m), 7.90 (2H, dd, J=5.6, 8.9Hz), 8.26 (1H, br  
30 d, J=7.7Hz)

MASS (APCI)(m/z): 313

Reference preparation 8

The following compound was obtained by using  
4-amino-1-benzylpiperidine as a starting compound according to a  
35 similar manner to that of Reference example 2.

N-(1-Benzylpiperidin-4-yl)-N'-(4-fluorophenyl)urea

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.25-1.5 (2H, m), 1.7-1.9 (2H, m), 2.0-2.2 (2H, m), 2.65-2.8 (2H, m), 3.4-3.6 (3H, m), 6.07 (1H, d, J=7.6Hz),

7.05 (2H, t, J=9Hz), 7.2-7.45 (2H, m), 8.35 (1H, s)

MASS (APCI)(m/z): 328

Reference preparation 9

To a solution of N-(1-benzylpiperidin-4-yl)-N'-(4-fluoro-  
5 phenyl)urea (3.0 g) in a mixture of methanol (15 ml) and tetrahydrofuran  
(15 ml) was added palladium on carbon (10% w/w, 50% wet, 0.6 g), and  
the mixture was hydrogenated under atmospheric pressure of hydrogen  
for 8 hours. The catalyst was filtered off, and the solvents were  
evaporated under reduced pressure to give a residue, which was triturated  
10 with diisopropyl ether to give N-(piperidin-4-yl)-N'-  
(4-fluorophenyl)urea (1.97 g).

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.1-1.4 (2H, m), 1.65-1.85 (2H, m), 2.3-2.65 (2H,  
m), 2.8-3.0 (2H, m), 3.3-3.7 (1H, m), 6.08 (1H, d, J=8Hz), 7.04  
(2H, t, J=9Hz), 7.25-7.5 (2H, m), 8.33 (1H, s)

15 MASS (APCI)(m/z): 238

Reference preparation 10

A mixture of N-(1-benzylpiperidin-4-yl)-4-fluorobenzamide (937  
mg) and 10% palladium on carbon (50% wet, 0.2 g) in methanol (20 ml)  
was stirred under hydrogen atmosphere for 7.5 hours at ambient  
20 temperature. The catalyst was removed by glass filter and the solvent  
was removed under reduced pressure. After rinse with diisopropyl ether,  
N-(piperidin-4-yl)-4-fluorobenzamide (653 mg) was obtained.

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.40 (2H, ddd, J=4.0, 11.9, 23.8Hz), 1.72 (2H,  
br d, J=9.5Hz), 2.3-2.7 (2H, m), 2.8-3.2 (2H, m), 3.80 (1H, m),  
25 7.27 (2H, t, J=8.9Hz), 7.92 (2H, dd, J=5.6, 8.9Hz), 8.26 (1H,  
d, J=7.7Hz)

MASS (APCI)(m/z): 223

Reference example 1

To a solution of O-phenyl N-(4-pyridyl)carbamate (446 mg) in  
30 1,2-dichloroethane (5 ml) was added a suspension of 1-acetylpiperazine  
(1.12 g) in 1,2-dichloroethane (20 ml) at ambient temperature. The  
mixture was heated at 60°C with stirring for 9 hours. The mixture was  
cooled to ambient temperature, and diluted with dichloromethane and  
water. The aqueous phase was separated and adjusted to pH 11.5 with  
35 sodium hydroxide solution. Excess sodium chloride was added to the  
aqueous solution. The mixture was extracted with a mixture of  
dichloromethane and methanol (about 10:1) and the organic phase was  
washed with brine. After drying with magnesium sulfate, the solvents

were removed under reduced pressure. A residue was purified by column chromatography (silica gel 100 ml, dichloromethane:methanol:aqueous ammonia = 10:1:0.1). After rinse with diisopropyl ether, 1-acetyl-4-(4-pyridylaminocarbonyl)piperazine (398 mg) was obtained.

5 NMR (DMSO- $d_6$ ,  $\delta$ ): 2.03 (3H, s), 3.3-3.6 (8H, m), 7.47 (2H, dd, J=1.5, 4.8Hz), 8.31 (2H, dd, J=1.5, 4.8Hz), 9.01 (1H, s)

MASS (APCI)(m/z): 271

#### Reference example 2

To a stirred solution of 1-acetylpiperazine (0.648 g) in tetrahydrofuran (10 ml) was added 4-fluorophenyl isocyanate (0.574 g) at ambient temperature. After stirring at ambient temperature for 1 hour, the solvent was removed by evaporation under reduced pressure, and the residue was triturated with diisopropyl ether to give 1-acetyl-4-(4-fluorophenylcarbamoyl)piperazine (1.25 g).

15 NMR (DMSO- $d_6$ ,  $\delta$ ): 2.03 (3H, s), 3.3-3.6 (8H, m), 7.07 (2H, t, J=9Hz), 7.46 (2H, dd, J=5, 9Hz), 8.61 (1H, s)

MASS (APCI)(m/z): 266

#### Reference example 3

The following compound was obtained by using 1-tert-butoxycarbonylpiperazine as a starting compound according to a similar manner to that of Reference example 2.

1-tert-Butoxycarbonyl-4-(4-fluorophenylcarbamoyl)piperazine

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.42 (9H, s), 3.25-3.5 (8H, m), 7.07 (2H, t, J=9Hz), 7.45 (2H, dd, J=5, 9Hz), 8.60 (1H, s)

25 MASS (LD)(m/z): 346.2

#### Reference example 4

To a solution of pyridine-4-carboxylic acid (1.0 g) and triethylamine (1.2 ml) in toluene (20 ml) was added diphenylphosphoryl azide (1.75 ml) at ambient temperature. The resulting mixture was heated to reflux for 30 minutes and cooled to 0°C. To the mixture was added 1-tert-butoxycarbonylpiperazine (1.51 g), and the mixture was allowed to heat to 90°C for 1 hour. After cooling to ambient temperature, the reaction mixture was taken up into ethyl acetate, washed in turn with water and brine, dried over magnesium sulfate, and evaporated under reduced pressure. The residue was chromatographed on silica gel (150 ml) eluting with 0-7% methanol in dichloromethane. Trituration with a mixture of diisopropyl ether and ethanol gave 1-tert-butoxycarbonyl-4-(pyridin-4-ylcarbamoyl)piperazine (0.66 g).

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.42 (9H, s), 3.25-3.5 (8H, m), 7.46 (2H, d, J=1.5, 5Hz), 8.30 (2H, d, J=1.5, 5Hz), 9.00 (1H, s)

MASS (LD)(m/z): 307.2

Reference example 5

5 To a suspension of 1-acetyl-4-aminopiperidine hydrochloride (0.4 g) in dichloromethane (5 ml) were added in turn pyridine (0.54 ml) and 4-fluorophenyl chloroformate (0.29 ml) at 0°C. The mixture was allowed to warm to ambient temperature and stirred for 1 hour, which was taken up into a mixture of water and ethyl acetate. The separated  
10 organic layer was washed in turn with hydrochloric acid (1N), aqueous sodium hydrogen carbonate, and brine, and dried over magnesium sulfate. Evaporation under reduced pressure gave a residue, which was triturated with diisopropyl ether to give 1-acetyl-4-(4-fluorophenoxy carbonyl-amino)piperidine (347 mg).

15 NMR (DMSO- $d_6$ ,  $\delta$ ): 1.15-1.55 (2H, m), 1.7-1.95 (2H, m), 2.00 (3H, s), 2.65-2.85 (1H, m), 3.0-3.25 (1H, m), 3.5-3.7 (1H, m), 3.7-3.9 (1H, m), 4.15-4.3 (1H, m), 7.05-7.3 (4H, m), 7.86 (1H, d, J=8Hz)

MASS (APCI)(m/z): 281

Reference example 6

20 To a suspension of 1-acetyl-4-aminopiperidine hydrochloride (715 mg) in dichloromethane (7 ml) were added diisopropylethylamine (1.83 ml) and a solution of 4-fluorobenzoyl chloride (0.83 mg) in dichloromethane (2 ml) at ambient temperature. After stirring for 6.5 hours, the reaction mixture was diluted with dichloromethane and washed  
25 with water, saturated aqueous sodium hydrogen carbonate, and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 50 ml, dichloromethane:methanol = 50:1 to 10:1). After rinse with diisopropyl ether, N-(1-acetylpiperidin-4-yl)-4-fluoro-  
30 benzamide (738 mg) was obtained.

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.40 (2H, m), 1.81 (2H, distorted t, J=12.4Hz), 2.01 (3H, s), 2.68 (1H, br t, J=11.4Hz), 3.13 (1H, br t, J=11.6Hz), 3.83 (1H, br t, J=13.9Hz), 4.01 (1H, m), 4.33 (1H, br d, J=13.7Hz), 7.29 (2H, t, J=8.9Hz), 7.92 (2H, dd, J=5.5, 8.8Hz), 8.31 (1H, d, J=7.7Hz)  
35

MASS (APCI)(m/z): 265

Reference example 7

To a suspension of 1-acetyl-4-aminopiperidine hydrochloride (536



mg) in dichloromethane (5 ml) were added isonicotinoyl chloride hydrochloride (534 mg) and diisopropylethylamine (1.05 ml) at ambient temperature. After stirring for 8 hours, the reaction mixture was poured into water and diluted with dichloromethane. The mixture was adjusted to pH 8.5 with 1N sodium hydroxide solution. Sodium chloride was added to the mixture and an organic phase was separated. The aqueous phase was extracted with dichloromethane and a combined organic phase was dried over magnesium sulfate. The solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 50 ml, dichloromethane:methanol = 10:1). After crystallization from diisopropyl ether:n-hexane, N-(1-acetylpiperidin-4-yl)-N-isonicotinamide (477 mg) was obtained.

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.4 (2H, m), 1.83 (2H, distorted t, J=11Hz), 2.01 (3H, s), 2.69 (1H, br t, J=11Hz), 3.14 (1H, br t, J=12Hz), 3.83 (1H, br d, J=14.1Hz), 4.03 (1H, m), 4.33 (1H, br d, J=13.1Hz), 7.75 (2H, dd, J=1.7, 4.4Hz), 8.62 (1H, d, J=7.5Hz), 8.72 (2H, dd, J=1.6, 4.4Hz)

MASS (APCI)(m/z): 248

#### Reference example 8

To a suspension of 1-acetyl-4-aminopiperidine hydrochloride (715 mg) in dichloromethane (7 ml) were added diisopropylethylamine (1.83 ml) and a solution of 4-fluorobenzenesulfonyl chloride (0.83 mg) in dichloromethane (2 ml) at ambient temperature. After stirring for 6.5 hours, the reaction mixture was diluted with dichloromethane and washed with water, saturated aqueous sodium hydrogen carbonate, and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 50 ml, dichloromethane:methanol = 50:1 to 20:1). After rinse with diisopropyl ether, N-(1-acetylpiperidin-4-yl)-4-fluorobenzenesulfonamide (859 mg) was obtained.

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.21 (2H, m), 1.54 (2H, m), 1.94 (3H, s), 2.66 (1H, br t, J=10.8Hz), 3.02 (1H, dt, J=2.9, 12.0Hz), 3.22 (1H, m), 3.64 (1H, br d, J=14.0Hz), 4.05 (1H, br d, J=13.2Hz), 7.44 (2H, t, J=8.9Hz), 7.8-8.0 (3H, m)

MASS (APCI)(m/z): 301

#### Reference example 9

To a solution of O-phenyl N-(4-pyridyl)carbamate (0.81 g) in chloroform (10 ml) were added 1-acetyl-4-aminopiperidine hydrochloride

(0.68 g) and triethylamine (1.06 ml) at ambient temperature. After stirring for 1 day, the mixture changed to a solution. The solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 100 ml, dichloromethane:methanol = 10:1 to 5:1, and silica gel 50 ml, dichloromethane:methanol:aqueous ammonia = 10:1:0.1). The solvents of desired fractions were removed under reduced pressure. A residue was dissolved with methanol (5 ml) and dichloromethane (5 ml), and 4N hydrogen chloride in dioxane (1.5 ml) was added to the solution. The solvents were removed under reduced pressure, and the residue was evaporated azeotropically with methanol. After crystallization from diisopropyl ether and n-hexane, N-(1-acetylpiperidin-4-yl)-N'-(4-pyridyl)urea (343 mg) was obtained.

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.1-1.6 (2H, m), 1.77 (2H, m), 2.01 (3H, s), 2.94 (1H, br t, J=10.4Hz), 3.22 (1H, br t, J=10.1Hz), 3.76 (2H, m), 4.05 (1H, d, J=13.6Hz), 7.60 (1H, d, J=7.8Hz), 7.83 (2H, d, J=6.8Hz), 8.52 (2H, d, J=7.1Hz), 11.21 (1H, s), 14.66 (1H, br s)

MASS (APCI)(m/z): 263

#### Reference example 10

To a suspension of 1-acetyl-4-aminopiperidine hydrochloride (536 mg) in dichloromethane (5 ml) were added 4-fluorophenyl isocyanate (375  $\mu$ l) and diisopropylethylamine (575  $\mu$ l) at ambient temperature. After stirring for 3 hours, the reaction mixture was diluted with dichloromethane. An organic phase was separated and an aqueous phase was extracted with dichloromethane. A combined organic phase was dried over magnesium sulfate and the solvents were removed under reduced pressure. After crystallization from diisopropyl ether and n-hexane, N-(1-acetylpiperidin-4-yl)-N'-(4-fluorophenyl)urea (448 mg) was obtained.

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.1-1.5 (2H, m), 1.80 (2H, distorted t, J=10Hz), 2.00 (3H, s), 2.77 (1H, br d, J=10.8Hz), 3.14 (1H, br d, J=11.1Hz), 3.5-3.9 (2H, m), 4.16 (1H, br d, J=13.2Hz), 6.15 (1H, d, J=7.6Hz), 7.05 (2H, t, J=8.9Hz), 7.40 (2H, dd, J=5.0, 9.2Hz), 8.37 (1H, s)

MASS (APCI)(m/z): 280

#### Reference example 11

To a solution of 4-(4-fluorobenzoylamino)piperidine (0.25 g) in dichloromethane (5 ml) were added in turn pyridine (0.14 ml) and methyl chloroformate (87  $\mu$ l) at 0°C. The mixture was allowed to warm

to ambient temperature and stirred for 1 hour. To the mixture was added N,N-dimethylaminopyridine (0.13 g) and allowed to stir for another 1 hour. The reaction mixture was taken up into a mixture of water and ethyl acetate. The separated organic layer was washed in turn with  
5 hydrochloric acid (1N), aqueous sodium hydrogen carbonate, and brine, and dried over magnesium sulfate. Evaporation under reduced pressure gave a residue, which was triturated with diisopropyl ether to give 4-(4-fluorobenzoylamino)-1-methoxycarbonylpiperidine (0.265 g).

10 NMR (DMSO- $d_6$ ,  $\delta$ ): 1.3-1.6 (2H, m), 1.75-1.9 (2H, m), 2.8-3.05 (2H, m), 3.60 (3H, s), 3.85-4.1 (2H, m), 7.29 (2H, t, J=9Hz), 7.90 (2H, dd, J=6, 9Hz), 8.30 (1H, d, J=8Hz)

MASS (APCI)(m/z): 281

#### Reference example 12

To a solution of 4-(4-fluorobenzoylamino)piperidine (0.25 g)  
15 in pyridine (5 ml) were added in turn 4-fluorobenzenesulfonyl chloride (0.219 g) and catalytic amount of N,N-dimethylaminopyridine at 0°C. The mixture was allowed to warm to ambient temperature and stirred for 1 hour, which was taken up into a mixture of water and dichloromethane. The separated organic layer was washed in turn with hydrochloric acid  
20 (1N), aqueous sodium hydrogen carbonate, and brine, and dried over magnesium sulfate. Evaporation under reduced pressure gave a residue, which was triturated with diisopropyl ether to give 4-(4-fluorobenzoylamino)-1-(4-fluorophenylsulfonyl)piperidine (0.38 g).

25 NMR (DMSO- $d_6$ ,  $\delta$ ): 1.45-1.7 (2H, m), 1.8-1.95 (2H, m), 2.35-2.55 (2H, m), 3.5-3.85 (3H, m), 7.28 (2H, t, J=9Hz), 7.50 (2H, t, J=9Hz), 7.75-7.95 (4H, m), 8.31 (1H, d, J=8Hz)

MASS (APCI)(m/z): 381

#### Reference example 13

30 To a solution of 4-(4-fluorobenzoylamino)piperidine (0.15 g) in dichloromethane (5 ml) were added in turn pyridine (82  $\mu$ l) and 4-trifluoromethoxybenzoyl chloride (106  $\mu$ l) at 0°C. The mixture was allowed to warm to ambient temperature and stirred for 4 hours, which was taken up into a mixture of water and dichloromethane. The separated  
35 organic layer was washed in turn with hydrochloric acid (1N), aqueous sodium hydrogen carbonate, and brine, and dried over magnesium sulfate. Evaporation of the solvent under reduced pressure gave 4-(4-fluorobenzoylamino)-1-(4-trifluoromethoxybenzoyl)piperidine

(205 mg).

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.3-1.7 (2H, m), 1.7-2.0 (2H, m), 2.7-3.4 (2H, m), 3.4-3.8 (1H, m), 3.9-4.2 (1H, m), 4.2-4.6 (1H, m), 7.30 (2H, t, J=9Hz), 7.35-7.6 (4H, m), 7.91 (2H, dd, J=6, 9Hz), 8.35 (1H, d, J=8Hz)

MASS (LD)(m/z): 433.2

Reference example 14

To a solution of 4-(4-fluorobenzoylamino)piperidine (0.15 g) in dichloromethane (5 ml) were added in turn pyridine (0.14 ml) and methanesulfonyl chloride (96  $\mu$ l) at 0°C. The mixture was allowed to warm to ambient temperature and stirred for 1 hour. To the mixture was added N,N-dimethylaminopyridine (0.13 g) and allowed to stir for another 1 hour. The reaction mixture was taken up into a mixture of water and dichloromethane. The separated organic layer was washed in turn with hydrochloric acid (1N), aqueous sodium hydrogen carbonate, and brine, and dried over magnesium sulfate. Evaporation under reduced pressure gave a residue, which was triturated with diisopropyl ether to give 4-(4-fluorobenzoylamino)-1-methylsulfonylpiperidine (0.30 g).

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.45-1.7 (2H, m), 1.8-2.05 (2H, m), 2.7-2.95 (2H, m), 2.88 (3H, s), 3.5-3.65 (2H, m), 3.8-4.05 (1H, m), 7.30 (2H, t, J=9Hz), 7.91 (2H, dd, J=6, 9Hz), 8.36 (1H, d, J=8Hz)

MASS (APCI)(m/z): 301

Reference example 15

To a solution of N-(piperidin-4-yl)-N'-(4-fluorophenyl)-urea (0.3 g) in tetrahydrofuran (4 ml) were added in turn pyridine (0.28 ml), methyl chloroformate (98  $\mu$ l) and catalytic amount of N,N-dimethylaminopyridine at 0°C. The mixture was allowed to warm to ambient temperature and stirred for 2 hours. The reaction mixture was taken up into a mixture of water and ethyl acetate. The separated organic layer was washed in turn with hydrochloric acid (1N), aqueous sodium hydrogen carbonate, and brine, and dried over magnesium sulfate. Evaporation under reduced pressure gave a residue, which was triturated with diisopropyl ether to give N-(1-methoxycarbonylpiperidin-4-yl)-N'-(4-fluorophenyl)urea (0.312 g).

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.1-1.4 (2H, m), 1.7-1.9 (2H, m), 2.8-3.1 (2H, m), 3.5-3.75 (1H, m), 3.59 (3H, s), 3.75-3.95 (2H, m), 6.15 (1H, d, J=7.6Hz), 7.05 (2H, t, J=9Hz), 7.37 (2H, dd, J=5, 9Hz), 8.37

(1H, s)

MASS (APCI)(m/z): 296

Reference example 16

To a solution of N-(piperidin-4-yl)-N'-(4-fluorophenyl)urea (0.3 g) in tetrahydrofuran (4 ml) were added in turn N,N-dimethylamino-pyridine (0.23 g) and 4-fluorobenzenesulfonyl chloride (0.25 g) at 0°C. The mixture was allowed to warm to ambient temperature and stirred for 1 hour. The reaction mixture was taken up into a mixture of water and dichloromethane. The separated organic layer was washed in turn with hydrochloric acid (1N), aqueous sodium hydrogen carbonate, and brine, and dried over magnesium sulfate. Evaporation under reduced pressure gave a residue, which was triturated with diisopropyl ether to give N-(1-(4-fluorophenylsulfonyl)piperidin-4-yl)-N'-(4-fluorophenyl)urea (0.468 g).

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.3-1.6 (2H, m), 1.75-1.95 (2H, m), 2.45-2.7 (2H, m), 3.35-3.6 (3H, m), 6.14 (1H, d, J=7.5Hz), 7.03 (2H, t, J=9Hz), 7.34 (2H, dd, J=5, 9Hz), 7.50 (2H, t, J=9Hz), 7.75-7.95 (2H, m), 8.31 (1H, s)

MASS (APCI)(m/z): 396

Reference example 17

To a suspension of N-(piperidin-4-yl)-4-fluorobenzamide (0.5 g) in dichloromethane (5 ml) were added pyridine (218  $\mu$ l), dichloromethane (5 ml) and benzoyl chloride (290  $\mu$ l) at ambient temperature. After stirring for 3.5 hours, water (5 ml) was poured into the mixture. An organic layer was separated, and washed with water and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel, toluene:ethyl acetate = 1:1 to ethyl acetate). After rinse with diisopropyl ether,

N-(1-benzoylpiperidin-4-yl)-4-fluorobenzamide (515 mg) was obtained.

NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.50 (2H, br s), 1.85 (2H, br s), 2.8-3.3 (2H, m), 3.61 (1H, m), 4.1 (1H, m), 4.35 (1H, m), 7.29 (2H, t, J=8.9Hz), 7.3-7.5 (5H, m), 7.92 (2H, dd, J=5.6, 8.9Hz), 8.34 (1H, d, J=7.9Hz)

MASS (APCI)(m/z): 327

Reference example 18

To a suspension of N-(piperidin-4-yl)-4-fluorobenzamide (556 mg) in dichloromethane (5 ml) were added pivaloyl chloride (0.37 ml), pyridine (0.24 ml) and N,N-dimethylaminopyridine (25 mg) at ambient

temperature. After stirring for 1 day, the mixture was diluted with dichloromethane, and washed with water and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. After trituration with diisopropyl ether, N-(1-pivaloylpiperidin-4-

5 yl)-4-fluorobenzamide (305 mg) was obtained.

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.20 (9H, s), 1.41 (2H, m), 1.7-1.9 (2H, m), 2.91 (2H, br t,  $J=11.9\text{Hz}$ ), 4.07 (1H, m), 4.27 (2H, br d,  $J=13.3\text{Hz}$ ), 7.29 (2H, t,  $J=8.9\text{Hz}$ ), 7.92 (2H, dd,  $J=5.5, 8.9\text{Hz}$ ), 8.30 (1H, d,  $J=7.8\text{Hz}$ )

10 MASS (APCI)( $m/z$ ): 329

Reference example 19

To a suspension of N-(piperidin-4-yl)-4-fluorobenzamide (556 mg) in dichloromethane (6 ml) were added cyclopropanecarboxylic acid (0.20 ml), 1-hydroxybenzotriazole (338 mg) and 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide hydrochloride (480 mg) at ambient temperature. After stirring for 21 hours, the mixture was diluted with dichloromethane, and washed with water, saturated aqueous sodium hydrogen carbonate, and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. After crystallization from diisopropyl ether, N-(1-cyclopropylcarbonylpiperidin-4-yl)-4-fluorobenzamide (627 mg) was obtained.

NMR (DMSO- $d_6$ ,  $\delta$ ): 0.6-0.8 (4H, m), 1.2-1.6 (2H, m), 1.7-2.0 (2H, m), 1.85 (1H, m), 2.72 (1H, m), 3.21 (1H, m), 4.04 (1H, m), 4.30 (2H, m), 7.29 (2H, t,  $J=8.9\text{Hz}$ ), 7.92 (2H, dd,  $J=5.6, 8.9\text{Hz}$ ), 8.31 (1H, d,  $J=7.7\text{Hz}$ )

25 MASS (APCI)( $m/z$ ): 313

Reference example 20

1-tert-Butoxycarbonyl-4-(4-fluorophenylcarbamoyl)piperazine (0.30 g) was dissolved in a solution of hydrogen chloride in ethyl acetate (4N, 2 ml), and the solution was stirred at ambient temperature for 1 hour. The solvent was removed by evaporation under reduced pressure to give 1-(4-fluorophenylcarbamoyl)piperazine as a white powder, which was taken up into dichloromethane (3 ml), and to the mixture were added in turn pyridine (0.25 ml), 4-trifluoromethoxybenzoyl chloride (0.146 ml), and catalytic amount of N,N-dimethylaminopyridine. After stirring at ambient temperature for 12 hours, the mixture was washed in turn with hydrochloric acid (0.5N), aqueous sodium hydrogen carbonate, and brine, dried over magnesium

sulfate, and evaporated under reduced pressure. The residue was chromatographed on silica gel (50 ml) eluting with 0%-3% methanol in dichloromethane to give 1-(4-fluorophenylcarbamoyl)-4-(4-trifluoromethoxybenzoyl)piperazine (0.19 g).

5 NMR (DMSO- $d_6$ ,  $\delta$ ): 3.2-3.8 (8H, m), 7.08 (2H, t,  $J=9\text{Hz}$ ), 7.35-7.5 (4H, m), 7.5-7.65 (2H, m)

MASS (LD)( $m/z$ ): 434.1

#### Reference example 21

10 The following compound was obtained by using methyl chloroformate as a reactive derivative at the carboxy group according to a similar manner to that of Reference example 20.

1-Methoxycarbonyl-4-(4-fluorophenylcarbamoyl)piperazine

NMR (DMSO- $d_6$ ,  $\delta$ ): 3.3-3.5 (8H, m), 3.62 (3H, s), 7.07 (2H, t,  $J=9\text{Hz}$ ), 7.44 (2H, dd,  $J=5, 9\text{Hz}$ ), 8.62 (1H, s)

15 MASS (APCI)( $m/z$ ): 282

#### Reference example 22

20 A mixture of N-acetylpiperidine-4-carboxylic acid (514 mg), 1-hydroxybenzotriazole (405 mg), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (575 mg) and 4-fluoroaniline (284.2 ml) indichloromethane (5ml) was stirred for 18 hours at ambient temperature. The mixture was diluted with dichloromethane and washed with water, saturated aqueous sodium hydrogen carbonate, water, and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 25 40 ml, dichloromethane:methanol = 15:1). After trituration with diisopropyl ether, 1-acetyl-4-(4-fluorophenyl)carbamoylpiperidine (532 mg) was obtained.

30 NMR (DMSO- $d_6$ ,  $\delta$ ): 1.3-1.7 (2H, m), 1.8 (2H, m), 2.01 (3H, s), 2.5 (2H, m), 3.05 (1H, br t,  $J=10.6\text{Hz}$ ), 3.87 (1H, br d,  $J=14.1\text{Hz}$ ), 4.40 (1H, br d,  $J=13.1\text{Hz}$ ), 7.12 (2H, t,  $J=8.9\text{Hz}$ ), 7.61 (2H, dd,  $J=5.1, 9.1\text{Hz}$ ), 9.96 (1H, s)

MASS (APCI)( $m/z$ ): 265

#### Reference example 23

35 A solution of 1-acetylpiperazine-4-sulfonyl chloride (0.91 g) in chloroform (10 ml) were added 4-fluoroaniline (0.38 ml) and triethylamine (0.56 ml) at ambient temperature. After stirring for 6 days, the solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 100 ml,

dichloromethane:methanol=19:1). After rinse with diisopropyl ether, 1-acetyl-4-(4-fluorophenyl)sulfamoylpiperazine (716 mg) was obtained.

5 NMR (CDCl<sub>3</sub>,  $\delta$ ): 1.97 (3H, s), 3.09 (4H, m), 3.37 (4H, m), 7.20 (4H, m), 10.00 (1H, s)

MASS (APCI)(m/z): 302

Reference example 24

To a solution of O-tert-butyl (1-acetylpiperidin-4-yl)carbamate (0.97 g) in N,N-dimethylformamide (10 ml) was added 60% sodium hydride (0.18 g) at ambient temperature. After stirring for 40 minutes, 4-fluorobenzyl bromide (0.6 ml) was added to the reaction mixture. After additional stirring for 4 hours, the reaction mixture was poured into a mixture of ethyl acetate (50 ml) and water (10 ml). An organic phase was separated and washed with water and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. A residue was purified by column chromatography (silica gel 100 ml, toluene:ethyl acetate = 1:1 to 1:2). After crystallization from diisopropyl ether and n-hexane, O-tert-butyl N-(4-fluorobenzyl)-N-(1-acetylpiperidin-4-yl)carbamate (922 mg) was obtained.

20 NMR (DMSO-d<sub>6</sub>,  $\delta$ ): 1.35 (9H, br s), 1.3-1.8 (4H, m), 1.95 (3H, s), 2.3-2.6 (1H, m), 2.97 (1H, m), 3.80 (1H, br d, J=15.2Hz), 4.0 (1H, m), 4.32 (2H, s), 4.2-4.6 (1H, m), 7.0-7.4 (4H, m)

MASS (APCI)(m/z): 295

Reference example 25

25 To a solution of O-tert-butyl N-(4-fluorobenzyl)-N-(1-acetylpiperidin-4-yl)carbamate (0.5 g) in dichloromethane (5 ml) was added 4N hydrogen chloride in dioxane (5 ml). The reaction mixture was diluted with diisopropyl ether and the precipitates were collected by filtration. After drying under reduced pressure, 1-acetyl-4-(4-fluorobenzyl)-aminopiperidine hydrochloride (409 mg) was obtained.

30 NMR (DMSO-d<sub>6</sub>+D<sub>2</sub>O,  $\delta$ ): 1.54 (2H, m), 2.02 (3H, s), 2.0-2.3 (2H, m), 2.4-2.7 (1H, m), 3.04 (1H, br t, J=12.1Hz), 3.29 (1H, m), 3.9 (1H, m), 4.17 (2H, s), 4.44 (1H, br d, J=13.6Hz), 7.27 (2H, t, J=8.9Hz), 7.66 (2H, br t, J=6.8Hz)

35 MASS (APCI)(m/z): 251

Reference example 26

To a solution of N-(1-acetylpiperidin-4-yl)-4-fluorobenzamide (529 mg) in N,N-dimethylformamide (5 ml) was added sodium hydride (0.1



g). After stirring for 45 minutes, methyl iodide (623 ml) was added to the solution. After stirring for 45 minutes, the mixture was diluted with ethyl acetate (100 ml) and water (50 ml). An organic phase was separated, and washed with water and brine. After drying with magnesium sulfate, the solvents were removed under reduced pressure. After trituration with diisopropyl ether, N-(1-acetylpiperidin-4-yl)-N-methyl-4-fluorobenzamide (248 mg) was obtained.

NMR (DMSO- $d_6$ ,  $\delta$ ): 1.65 (4H, m), 2.00 (3H, s), 2.78 (3H, s), 3.8 (1H, m), 4.4 (1H, m), 2.0-4.6 (3H, br m), 7.26 (2H, t,  $J=8.9$ Hz), 7.46 (2H, dd,  $J=5.6, 8.7$ Hz)

MASS (APCI)(m/z): 301

#### Reference example 27

A suspension of 1-acetylpiperazine (0.627 g), 2-chloro-4'-fluoroacetophenone (0.844 g), and potassium hydrogen carbonate (0.735 g) in acetonitrile (12 ml) was stirred at ambient temperature for 3 days. After removal of the solid by filtration, the filtrate was evaporated under reduced pressure to give a residue, which was chromatographed on silica gel (100 ml) eluting with 0%-5% methanol in dichloromethane. The objective compound of the free form was taken up into ethyl acetate (2 ml) and to the solution was added a solution of hydrogen chloride in ethyl acetate (4N, 2 ml). The resulting precipitate was collected by filtration, washed with diisopropyl ether, and dried in vacuo to give 1-acetyl-4-(4-fluorophenylcarbonylmethyl)-piperazine hydrochloride (1.47 g).

NMR (DMSO- $d_6$ ,  $\delta$ ): 2.06 (3H, s), 2.95-3.8 (6H, m), 3.9-4.15 (1H, m), 4.2-4.45 (1H, m), 5.13 (2H, s), 7.48 (2H, t,  $J=9$ Hz), 8.09 (2H, dd,  $J=5, 9$ Hz)

MASS (APCI)(m/z): 265

#### Industrial Applicability

An agent for expression of long-term potentiation of synaptic transmission comprising a compound having a brain somatostatin activation property of the present invention is effective for the prophylaxis and/or treatment of cerebral diseases such as dementia (e.g., senile dementia, Alzheimer's dementia, dementia associated with various diseases such as cerebral vascular dementia, cerebral post-traumatic dementia, dementia due to brain tumor, dementia due to chronic subdural hematoma, dementia due to normal pressure

hydrocephalus, post-meningitis dementia, Parkinson's disease type dementia, and the like), amnesia, manic-depressive psychosis, schizophrenia, Parkinson's disease, psychosomatic disease, and the like, particularly for the prophylaxis and/or treatment of dementia  
5 and amnesia. The present invention also relates to a screening method of an agent for the expression of long-term potentiation of synaptic transmission, which uses a somatostatin releasing property as an index. By using the present screening method, various compounds useful for the prophylaxis and/or treatment of the above-mentioned cerebral  
10 diseases can be selected.

This invention is based on application No. 09/321,745 filed in the United States of America, the content of which is incorporated hereinto by reference.

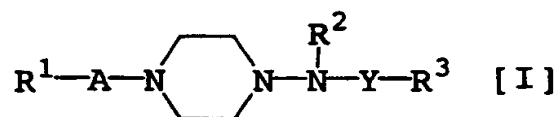
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## CLAIMS

1. An agent for expression of long-term potentiation of synaptic transmission, which comprises a compound having a brain somatostatin  
5 activation property as an active ingredient.

2. The agent for expression of long-term potentiation of synaptic transmission of claim 1, wherein the compound exerts an action to promote a release of brain somatostatin through suppression of a negative  
10 feedback mechanism of brain somatostatin release.

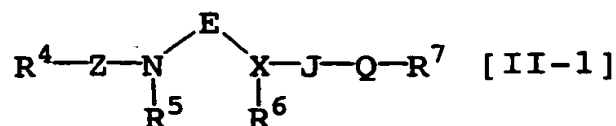
3. The agent for expression of long-term potentiation of synaptic transmission of claim 1 or claim 2, wherein the compound has the following  
15 formula [I]:



wherein

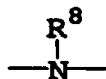
$\text{R}^1$  is lower alkyl, aryl, ar(lower)alkoxy or heterocyclic group, each of which may be substituted with halogen,  
20  $\text{R}^2$  is hydrogen atom or lower alkyl,  
 $\text{R}^3$  is cyclo(lower)alkyl, aryl or ar(lower)alkyl, each of which may be substituted with halogen,  
 $\text{A}$  is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or lower alkylene, and  
 $\text{Y}$  is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or  $\text{---CONH---}$ ,  
25 or pharmaceutically acceptable salts thereof.

4. The agent for expression of long-term potentiation of synaptic transmission of claim 1 or claim 2, wherein the compound has the following  
30 formula [II-1]:



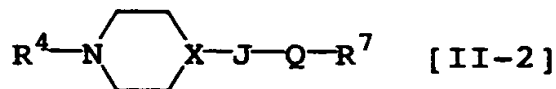
wherein

- $R^4$  is acyl,  
 $R^7$  is lower alkyl, lower alkoxy, lower alkylamino, lower alkenyl, lower alkenyloxy, lower alkenylamino, lower alkynyl, lower alkynyloxy, lower alkynylamino,  
 5 cyclo(lower)alkyl, cyclo(lower)alkyloxy, cyclo(lower)alkylamino, aryl, aryloxy, arylamino, a heterocyclic group or amino substituted with a heterocyclic group, each of which may be substituted with suitable substituent(s); or acyl;  
 10 Z is a single bond, -CO- or -SO<sub>2</sub>-,  
 E is lower alkylene optionally substituted with suitable substituent(s),  
 X is CH or N,  
 J is a single bond, lower alkylene or  
 15



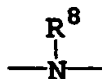
- wherein  $R^8$  is hydrogen, lower alkyl, substituted-lower alkyl, an N-protective group, aryl, acyl or a heterocyclic group,  
 Q is -CH<sub>2</sub>-, -CO-, -SO<sub>2</sub>- or -N=CH-, and  
 20  $R^5$  and  $R^6$  are each hydrogen or lower alkyl, or are taken together to form lower alkylene optionally condensed with a cyclic hydrocarbon or a heterocyclic ring,  
 provided that when X is N,  
 then 1) J is a single bond, and Q is -CH<sub>2</sub>-, -CO- or -SO<sub>2</sub>-, or  
 25 2) J is lower alkylene,  
 or pharmaceutically acceptable salts thereof.

5. The agent for expression of long-term potentiation of synaptic transmission of claim 1 or claim 2, wherein the compound has the following  
 30 formula [II-2]:



wherein

- $R^4$  is acyl,  
 $R^7$  is aryl, aryloxy or arylamino, the aryl moiety of all of which may be substituted with halogen; pyridyl; or pyridylamino;  
 5     $X$  is CH or N,  
        $J$  is a single bond, lower alkylene or



- wherein  $R^8$  is hydrogen, lower alkyl or an N-protective group,  
 10     $Q$  is  $-CH_2-$ ,  $-CO-$  or  $-SO_2-$ ,  
 provided that when  $X$  is N, then  $J$  is a single bond or lower alkylene, or pharmaceutically acceptable salts thereof.

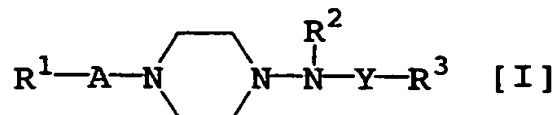
6. The agent for expression of long-term potentiation of synaptic transmission of any of claim 1 to claim 5, which is an agent for the prophylaxis or treatment of cerebral diseases.

7. The agent for expression of long-term potentiation of synaptic transmission of claim 6, which is an agent for the prophylaxis or treatment of dementia or amnesia.

8. A method for expressing long-term potentiation of synaptic transmission, comprising administering an effective amount of a compound having a brain somatostatin activation property.

9. The method for expressing long-term potentiation of synaptic transmission of claim 8, wherein the compound exerts an action to promote a release of brain somatostatin through suppression of a negative feedback mechanism of brain somatostatin release.

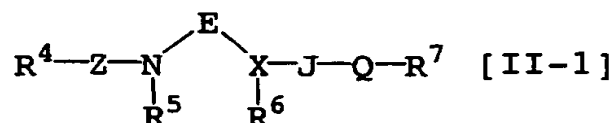
10. The method for expressing long-term potentiation of synaptic transmission of claim 8 or claim 9, wherein the compound has the following formula [I]:



wherein

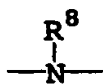
- $\text{R}^1$  is lower alkyl, aryl, ar(lower)alkoxy or heterocyclic group, each of which may be substituted with halogen,  
 $\text{R}^2$  is hydrogen atom or lower alkyl,  
 $\text{R}^3$  is cyclo(lower)alkyl, aryl or ar(lower)alkyl, each of which may be substituted with halogen,  
 $\text{A}$  is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or lower alkylene, and  
 $\text{Y}$  is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or  $\text{---CONH---}$ ,  
 or pharmaceutically acceptable salts thereof.

11. The method for expressing long-term potentiation of synaptic transmission of claim 8 or claim 9, wherein the compound has the following formula [II-1]:



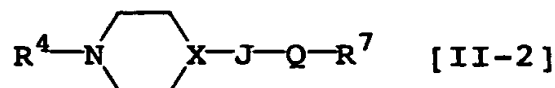
wherein

- $\text{R}^4$  is acyl,  
 $\text{R}^7$  is lower alkyl, lower alkoxy, lower alkylamino, lower alkenyl, lower alkenyloxy, lower alkenylamino, lower alkynyl, lower alkynyloxy, lower alkynylamino, cyclo(lower)alkyl, cyclo(lower)alkyloxy, cyclo(lower)alkylamino, aryl, aryloxy, arylamino,  
 a heterocyclic group or amino substituted with a heterocyclic group, each of which may be substituted with suitable substituent(s); or acyl;  
 $\text{Z}$  is a single bond,  $\text{---CO---}$  or  $\text{---SO}_2\text{---}$ ,  
 $\text{E}$  is lower alkylene optionally substituted with suitable substituent(s),  
 $\text{X}$  is CH or N,  
 $\text{J}$  is a single bond, lower alkylene or

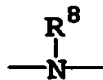


- wherein  $R^8$  is hydrogen, lower alkyl, substituted-lower alkyl, an N-protective group, aryl, acyl or a heterocyclic group,
- 5     $Q$  is  $-CH_2-$ ,  $-CO-$ ,  $-SO_2-$  or  $-N=CH-$ , and  
       $R^5$  and  $R^6$  are each hydrogen or lower alkyl, or are taken together to form lower alkylene optionally condensed with a cyclic hydrocarbon or a heterocyclic ring,
- provided that when  $X$  is  $N$ ,
- 10    then 1)  $J$  is a single bond, and  $Q$  is  $-CH_2-$ ,  $-CO-$  or  $-SO_2-$ , or  
         2)  $J$  is lower alkylene,  
      or pharmaceutically acceptable salts thereof.

12. The method for expressing long-term potentiation of synaptic transmission of claim 8 or claim 9, wherein the compound has the following formula [II-2]:
- 15



- wherein
- 20     $R^4$  is acyl,  
       $R^7$  is aryl, aryloxy or arylamino, the aryl moiety of all of which may be substituted with halogen; pyridyl; or pyridylamino;
- $X$  is  $CH$  or  $N$ ,
- 25     $J$  is a single bond, lower alkylene or



- wherein  $R^8$  is hydrogen, lower alkyl or an N-protective group,
- $Q$  is  $-CH_2-$ ,  $-CO-$  or  $-SO_2-$ ,
- 30    provided that when  $X$  is  $N$ , then  $J$  is a single bond or lower alkylene,

or pharmaceutically acceptable salts thereof.

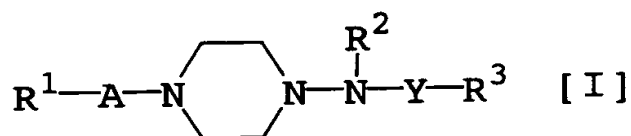
13. The method for expressing long-term potentiation of synaptic transmission of any of claim 8 to claim 12, which is a method for the prophylaxis or treatment of cerebral diseases.

14. The method for expressing long-term potentiation of synaptic transmission of claim 13, which is a method for the prophylaxis and/or treatment of dementia or amnesia.

15. Use of a compound having a brain somatostatin activation property for the production of an agent for the expression of long-term potentiation of synaptic transmission.

16. The use of a compound having a brain somatostatin activation property according to claim 15, wherein the compound exerts an action to promote a release of brain somatostatin through suppression of a negative feedback mechanism of brain somatostatin release.

17. The use of a compound having a brain somatostatin activation property according to claim 15 or claim 16, wherein the compound has the following formula [I]:



wherein

$\text{R}^1$  is lower alkyl, aryl, ar(lower)alkoxy or heterocyclic group, each of which may be substituted with halogen,

$\text{R}^2$  is hydrogen atom or lower alkyl,

$\text{R}^3$  is cyclo(lower)alkyl, aryl or ar(lower)alkyl, each of which may be substituted with halogen,

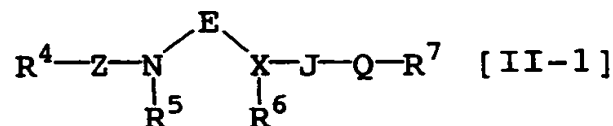
A is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or lower alkylene, and

Y is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or  $\text{---CONH---}$ ,

or pharmaceutically acceptable salts thereof.



18. The use of a compound having a brain somatostatin activation property according to claim 15 or claim 16, wherein the compound has the following formula [II-1]:



5

wherein

$\text{R}^4$  is acyl,

$\text{R}^7$  is lower alkyl, lower alkoxy, lower alkylamino, lower alkenyl, lower alkenyloxy, lower alkenylamino, lower alkynyl, lower alkynyloxy, lower alkynylamino,

10

cyclo(lower)alkyl, cyclo(lower)alkyloxy, cyclo(lower)alkylamino, aryl, aryloxy, arylamino, a heterocyclic group or amino substituted with a heterocyclic group, each of which may be substituted with suitable substituent(s); or acyl;

15

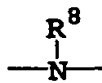
Z is a single bond, -CO- or -SO<sub>2</sub>-,

E is lower alkylene optionally substituted with suitable substituent(s),

X is CH or N,

20

J is a single bond, lower alkylene or



wherein  $\text{R}^8$  is hydrogen, lower alkyl, substituted-lower alkyl, an N-protective group, aryl, acyl or a heterocyclic group,

25

Q is -CH<sub>2</sub>-, -CO-, -SO<sub>2</sub>- or -N=CH-, and

$\text{R}^5$  and  $\text{R}^6$  are each hydrogen or lower alkyl, or are taken together to form lower alkylene optionally condensed with a cyclic hydrocarbon or a heterocyclic ring,

provided that when X is N,

30

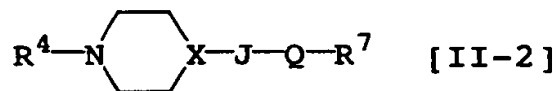
then 1) J is a single bond, and Q is -CH<sub>2</sub>-, -CO- or -SO<sub>2</sub>-, or

2) J is lower alkylene,

or pharmaceutically acceptable salts thereof.

19. The use of a compound having a brain somatostatin activation property according to claim 15 or claim 16, wherein the compound has the following formula [II-2]:

5



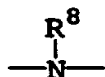
wherein

$R^4$  is acyl,

10  $R^7$  is aryl, aryloxy or arylamino, the aryl moiety of all of which may be substituted with halogen; pyridyl; or pyridylamino;

$X$  is CH or N,

$J$  is a single bond, lower alkylene or



15

wherein  $R^8$  is hydrogen, lower alkyl or an N-protective group,

$Q$  is  $-\text{CH}_2-$ ,  $-\text{CO}-$  or  $-\text{SO}_2-$ ,

provided that when  $X$  is N, then  $J$  is a single bond or lower alkylene, or pharmaceutically acceptable salts thereof.

20

20. The use of a compound having a brain somatostatin activation property according to any of claim 15 to claim 19, which is for the production of an agent for the prophylaxis and/or treatment of cerebral diseases.

25

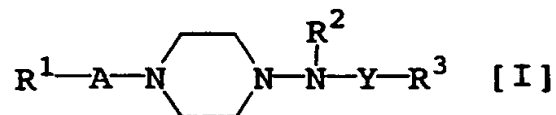
21. The use of a compound having a brain somatostatin activation property according to claim 20, which is for the production of an agent for the prophylaxis and/or treatment of dementia or amnesia.

30

22. A pharmaceutical composition for expression of long-term potentiation of synaptic transmission, which comprises a compound having a brain somatostatin activation property, and a pharmaceutically acceptable carrier or excipient.

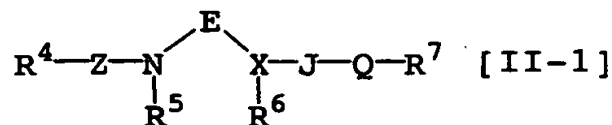
23. The pharmaceutical composition for expression of long-term  
 potentiation of synaptic transmission of claim 22, wherein the compound  
 exerts an action to promote a release of brain somatostatin through  
 suppression of a negative feedback mechanism of brain somatostatin  
 5 release.

24. The pharmaceutical composition for expression of long-term  
 potentiation of synaptic transmission of claim 22 or claim 23, wherein  
 the compound has the following formula [I]:  
 10



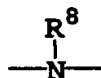
wherein  
 $\text{R}^1$  is lower alkyl, aryl, ar(lower)alkoxy or heterocyclic  
 group, each of which may be substituted with halogen,  
 15  $\text{R}^2$  is hydrogen atom or lower alkyl,  
 $\text{R}^3$  is cyclo(lower)alkyl, aryl or ar(lower)alkyl, each of which  
 may be substituted with halogen,  
 $\text{A}$  is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or lower alkylene, and  
 $\text{Y}$  is  $\text{---CO---}$ ,  $\text{---SO}_2\text{---}$  or  $\text{---CONH---}$ ,  
 20 or pharmaceutically acceptable salts thereof.

25. The pharmaceutical composition for expression of long-term  
 potentiation of synaptic transmission of claim 22 or claim 23, wherein  
 the compound has the following formula [II-1]:  
 25



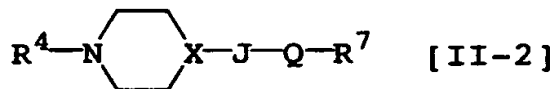
wherein  
 $\text{R}^4$  is acyl,  
 30  $\text{R}^7$  is lower alkyl, lower alkoxy, lower alkylamino, lower  
 alkenyl, lower alkenyloxy, lower alkenylamino, lower  
 alkynyl, lower alkynyloxy, lower alkynylamino,

- cyclo(lower)alkyl, cyclo(lower)alkyloxy,  
 cyclo(lower)alkylamino, aryl, aryloxy, arylamino,  
 a heterocyclic group or amino substituted with  
 a heterocyclic group, each of which may be substituted with  
 suitable substituent(s); or acyl;
- 5        Z        is a single bond, -CO- or -SO<sub>2</sub>-,  
        E        is lower alkylene optionally substituted with suitable  
                  substituent(s),  
        X        is CH or N,  
 10      J        is a single bond, lower alkylene or



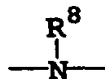
- wherein R<sup>8</sup> is hydrogen, lower alkyl, substituted-lower alkyl,  
 an N-protective group, aryl, acyl or a heterocyclic group,
- 15      Q        is -CH<sub>2</sub>-, -CO-, -SO<sub>2</sub>- or -N=CH-, and  
        R<sup>5</sup> and R<sup>6</sup> are each hydrogen or lower alkyl, or are taken together  
                  to form lower alkylene optionally condensed with a cyclic  
                  hydrocarbon or a heterocyclic ring,  
        provided that when X is N,  
 20      then 1) J is a single bond, and Q is -CH<sub>2</sub>-, -CO- or -SO<sub>2</sub>-, or  
               2) J is lower alkylene,  
        or pharmaceutically acceptable salts thereof.

26. The pharmaceutical composition for expression of long-term  
 25      potentiation of synaptic transmission of claim 22 or claim 23, wherein  
        the compound has the following formula [II-2]:



- wherein
- 30      R<sup>4</sup>        is acyl,  
        R<sup>7</sup>        is aryl, aryloxy or arylamino, the aryl moiety of all of  
                  which may be substituted with halogen; pyridyl; or  
                  pyridylamino;

X is CH or N,  
J is a single bond, lower alkylene or



- 5                    wherein  $R^8$  is hydrogen, lower alkyl or an N-protective group,  
Q                    is  $-CH_2-$ ,  $-CO-$  or  $-SO_2-$ ,  
provided that when X is N, then J is a single bond or lower alkylene,  
or pharmaceutically acceptable salts thereof.
- 10    27. The pharmaceutical composition for expression of long-term  
potentiation of synaptic transmission of any of claim 22 to claim 26,  
which is a pharmaceutical composition for the prophylaxis or treatment  
of cerebral diseases.
- 15    28. The pharmaceutical composition for expression of long-term  
potentiation of synaptic transmission of claim 27, which is a  
pharmaceutical composition for the prophylaxis or treatment of dementia  
or amnesia.
- 20    29. A method for screening an agent for expression of long-term  
potentiation of synaptic transmission, which comprises using a  
somatostatin releasing action as an index.
- 25    30. The screening method of claim 29, which is a screening method of  
an anti-dementia agent or anti-amnesia agent.
- 30    31. A method for screening an agent for expression of long-term  
potentiation of synaptic transmission, which comprises stimulating  
hippocampal slices, bringing a hippocampal slice into contact with  
a test compound, measuring an amount of somatostatin released from  
the hippocampal slice and/or a release time thereof, measuring an amount  
of somatostatin released from a hippocampal slice and/or a release  
time thereof in the absence of a contact with the test compound, and  
comparing the amounts and/or the times to calculate the amount of  
35    somatostatin released from the hippocampal slice and/or the release

time thereof caused by the contact with the test compound.

32. The screening method according to claim 31, which is a screening method of an anti-dementia agent or anti-amnesia agent.

5

33. The agent for expression of long-term potentiation of synaptic transmission of claim 1, wherein the compound having the brain somatostatin activation property is a compound obtained by the screening method of any of claim 29 to claim 32.

10

34. The method for expressing long-term potentiation of synaptic transmission according to claim 8, wherein the compound having the brain somatostatin activation property is a compound obtained by the screening method of any of claim 29 to claim 32.

15

35. The use of a compound having a brain somatostatin activation property according to claim 15, wherein the compound having the brain somatostatin activation property is obtained by the screening method of any of claim 29 to claim 32.

20

36. The pharmaceutical composition for expression of long-term potentiation of synaptic transmission of claim 22, wherein the compound having the brain somatostatin activation property is a compound obtained by the screening method of any of claim 29 to claim 32.

25

37. A commercial package comprising the pharmaceutical composition for expression of long-term potentiation of synaptic transmission of any of claim 22 to claim 28 or claim 36 and a written matter associated therewith, wherein the written matter states that the pharmaceutical composition can or should be used for expression of long-term potentiation of synaptic transmission.

30

38. A compound selected by the screening method described in any of claim 29 to claim 32.

35

FIG.1

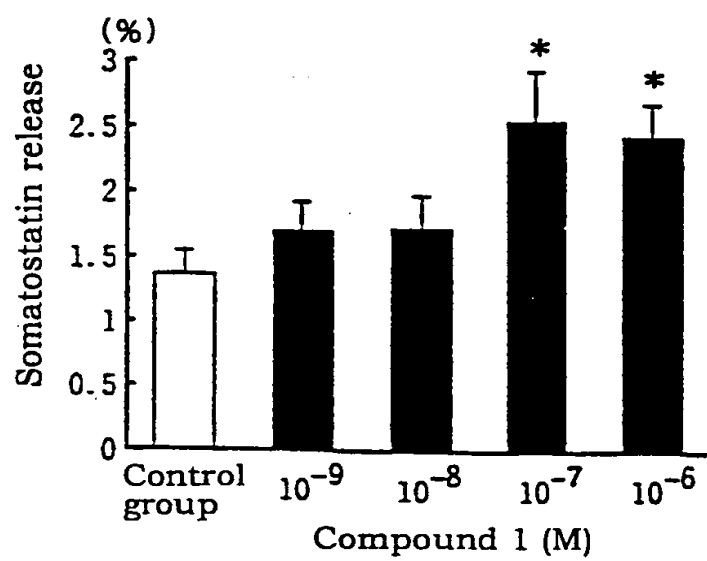


FIG.2

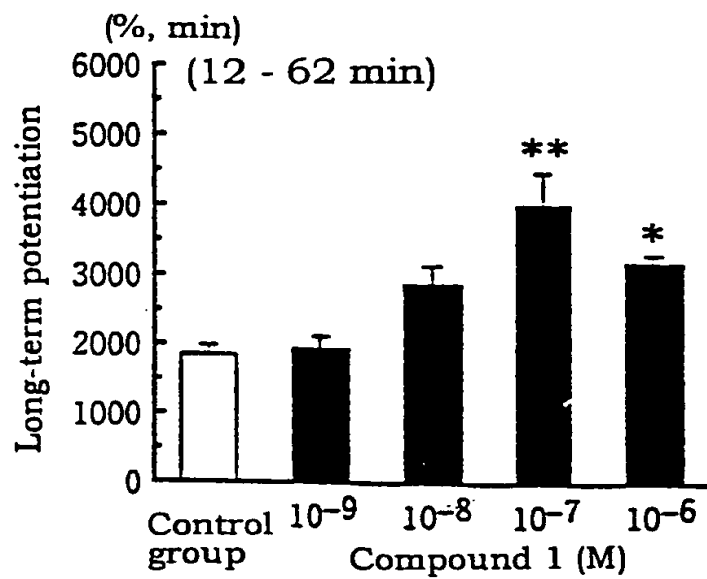




FIG.3

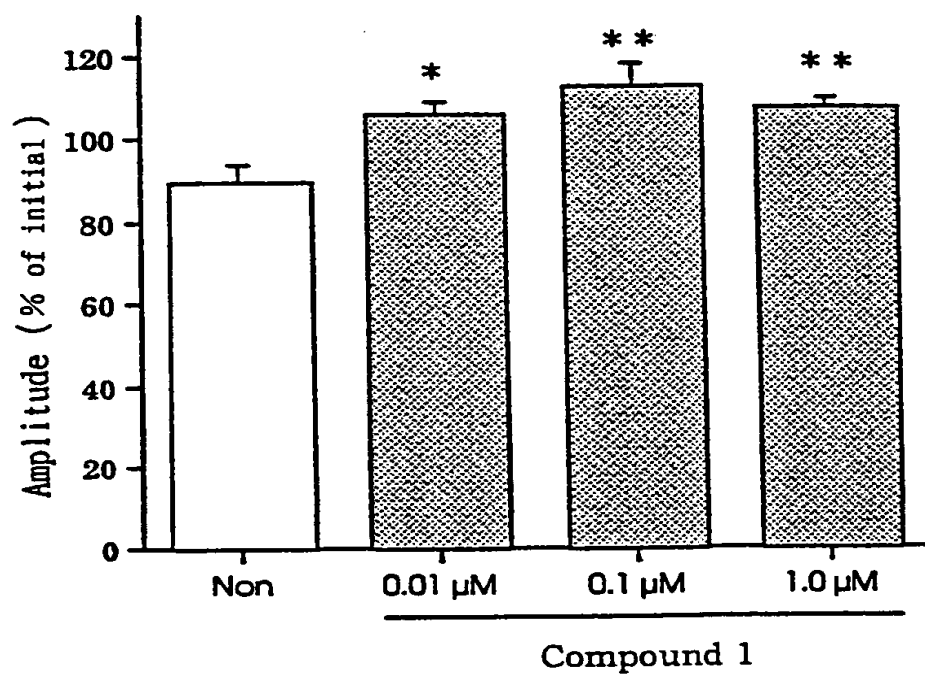
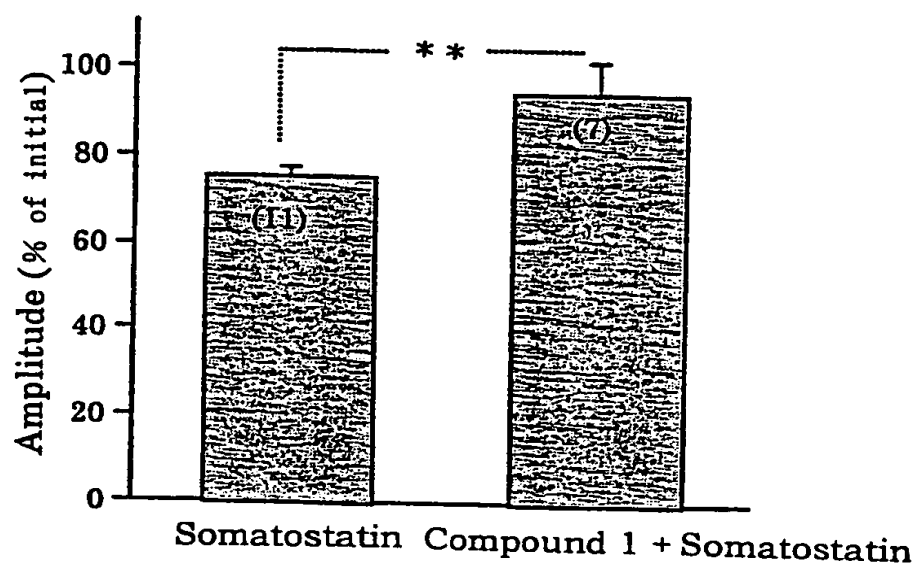


FIG.4



09/926,641

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8514 (JP).(88) Date of publication of the international search report:  
11 July 2002

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ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

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(54) Title: AGENT FOR EXPRESSION OF LONG-TERM POTENTIATION OF SYNAPTIC TRANSMISSION COMPRISING  
COMPOUND HAVING BRAIN SOMATOSTATIN ACTIVATION PROPERTY(57) Abstract: The present invention relates to an agent for the expression of long-term potentiation of synaptic transmission, which  
contains a compound having a brain somatostatin activation property as an active ingredient and to a screening method of an agent  
for the expression of long-term potentiation of synaptic transmission, which uses a somatostatin releasing property as an index.  
The present invention is useful for the prophylaxis and/or treatment of cerebral diseases of dementia, amnesia, manic-depressive  
psychosis, schizophrenia, Parkinson's disease, psychosomatic disease and the like.

WO 00/072834 A3

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/JP 00/03334

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/495 A61K31/4468 A61K31/4545 G01N33/15

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal, BIOSIS, MEDLINE, EMBASE, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.                                |
|------------|--|--|
| X          | MATSUOKA, NOBUYA ET AL: "FK960<br>[N-(4-acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate], a novel potential<br>antidementia drug, improves visual<br>recognition memory in rhesus monkeys:<br>comparison with physostigmine"<br>J. PHARMACOL. EXP. THER. (1997), 280(3),<br>1201-1209,<br>1997, XP001015389<br>abstract<br>---<br>-/-- | 1-3,<br>6-10,<br>13-17,<br>20-24,<br>27,28,<br>33-38 |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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Date of the actual completion of the international search

7 September 2001

Date of mailing of the international search report

27. 12. 01

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## INTERNATIONAL SEARCH REPORT

International Application No

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.   |
|------------|--|---|
| X          | <p>GRAUL, A. ET AL: "FK-960. Cognition enhancer"<br/>           DRUGS FUTURE (1997), 22(8), 830-832,<br/>           1997, XP001015394</p> <p>page 830, column 2, paragraph 3 -page 832,<br/>           column 1, paragraph 2</p> <p>---</p>  | <p>1-3,<br/>           6-10,<br/>           13-17,<br/>           20-24,<br/>           27,28,<br/>           33-38</p> |
| X          | <p>WO 98 25914 A (BABA YUKIHISA ;MIMURA<br/>           HISASHI (JP); FUJISAWA PHARMACEUTICAL CO<br/>           (JP) 18 June 1998 (1998-06-18)</p> <p>page 1, line 1-22; claims 1,6,7</p> <p>---</p>  | <p>1-3,<br/>           6-10,<br/>           13-17,<br/>           20-24,<br/>           27,28,<br/>           33-38</p> |
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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/JP 00/03334

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.  |
|------------|---|--|
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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/JP 00/03334

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-28, 33-38

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-28,33-38

Agents of formulae (I), (II-1) or (II-2), pharmaceutical compositions containing them, and use thereof for treating dementia or amnesia.

2. Claims: 29-32

A screening method for agents with anti-dementia or anti-amnesia properties comprises using a somatostatin releasing action as an index.



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/JP 00/03334

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s)  | Publication<br>date       |
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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/JP 00/03334

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
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